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(54) Title: BAND 3 ANTIGENIC PEPTIDES, MALARIA POLYPEPTIDES AND USES THEREOF

(57) Abstract: The invention provides peptides derived from erythroid Band 3 protein, which selectively bind to merozoite surface protein-1 (MSP-1), and/or one or more of the malaria polypeptides: BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA and prevent infection by the parasite of a Band 3-expressing cell, such as an erythrocyte. The invention also provides the isolated polypeptides BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA as well as peptides derived from MSP-1, which selectively bind to erythroid Band 3 protein and prevent parasite invasion into a Band 3-expressing cell, and prevent *Plasmodium* infection. Methods of using the malaria and MSP1 polypeptides of the invention for malaria prevention and/or treatment (e.g. in vaccines) are also provided. Antibodies that bind to the Band 3 polypeptides and anti-idiotypic antibodies thereto also are provided. Methods for selecting agents which inhibit Band 3-mediated parasite entry into target cells and methods of treatment which involve the polypeptides, antibodies, and anti-idiotypic antibodies also are provided.

BAND 3 ANTIGENIC PEPTIDES, MALARIA POLYPEPTIDES AND USES THEREOF

Related Applications

This application claims priority under 35 U.S.C. §119 to U.S. 60/272,930, filed March 2, 2001, the entire contents of which is hereby incorporated by reference.

Government Support

This invention was made in part with government support under grant number HL60961 and HL60755 from the National Institutes of Health (NIH). The government may have certain rights in this invention.

Field of the Invention

This invention relates to polypeptides derived from erythroid Band 3 protein and nucleic acid molecules encoding same. The polypeptides selectively bind to merozoite surface protein-1 (MSP-1) and/or to one or more of the polypeptides: BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA. The invention also relates in part to nucleic acids that encode the polypeptides BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA. The invention also relates to polypeptides derived from MSP-1 which selectively bind to Band 3 protein and nucleic acid molecules encoding same. The nucleic acid molecules and encoded polypeptides are useful in, *inter alia*, research, diagnostic and therapeutic contexts, particularly for the development of antibodies and anti-idiotypic antibodies for treating malaria infection.

Background of the Invention

The World Health Organization estimates that 300-500 million people are infected by malaria annually and over 2 million people, mostly women and children under the age of five die of the malaria disease each year. The disease has been classified as an "emerging infection" by many national and international health authorities in recent years, due to its dramatic comeback in regions where the disease is once eliminated or suppressed. Conventional method of control for malaria disease

mainly relies on the use of antimalarial drugs. Due to a rapid rise and spread of drug resistance to most affordable and widely used drugs in recent years, however, there is unfortunately limited means of treatment for the disease. At present, a malaria vaccine is not available.

In view of the foregoing, a need exists to develop new and improved methods and compositions for treating malaria infection. Preferably such methods and compositions are based upon inhibiting the particular interactions between the malaria parasite and a cognate molecule present in the host, thereby minimizing harmful side effects that may be due to non-specific therapeutic approaches.

Summary of the Invention

The invention is based, in part, on our discovery that the erythrocyte Band 3 protein is an important receptor for malaria parasite invasion into host erythrocytes. Important regions of the Band 3 protein that form the receptor in human erythrocytes are defined as amino acid residues 720-761 in the ectoplasmic domain 5 and residues 807-826 in the ectoplasmic domain 6. These two ectoplasmic domains of the erythroid Band 3 protein appear to be an important part of the erythrocyte receptor or receptor complex required for the *P. falciparum* invasion of the erythrocytes.

It now has been discovered that particular sequences within the erythroid Band 3 protein (also known as Anion Exchanger 1 or AE1) selectively interact with merozoite surface protein-1 (MSP-1) protein, resulting in entry of the malaria parasite into the erythrocyte host cell. In addition, other polypeptides that selectively interact with the Band-3 derived sequences have been identified. These polypeptides include: BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA. Accordingly, these particular Band 3-derived sequences are useful for further defining the nature of the interaction(s) between the parasite and the erythrocyte which result in infection, as well as for developing diagnostic and therapeutic agents which are useful for detecting and treating malaria infection. The knowledge of the particular sequences of the Band 3 protein which are important to malaria infection also permits the development of novel anti-idiotypic agents for treating malaria infection. These aspects of the invention are summarized below.

The invention also is based, in part, on the discovery that the polypeptides BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, ABRA, and particular sequences within MSP-1 selectively interact with Band 3 protein. Accordingly, the polypeptides BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, ABRA and these particular MSP-1 sequences are useful as targets for developing diagnostic and therapeutic agents for detecting and treating malaria infection. These aspects of the invention are summarized below.

In view of the foregoing discoveries, the invention embraces methods for inhibiting the selective interaction between the Band 3 protein and one or more of the polypeptides BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, ABRA, and MSP-1, as well as related compositions. Such methods are useful for identifying compounds for therapeutic use (e.g., screening assays), as well as for diagnosing and/or treating a malaria infection.

In addition, the invention also relates in part to nucleic acids that encode the polypeptides BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, ABRA and MSP-1.

According to one aspect of the invention, isolated Band 3 polypeptides are provided. The isolated Band 3 polypeptides include amino acid sequences selected from the group consisting of SEQ ID NO. 1, 2, 3, and 4 as shown herein: SEQ ID NO:1: GMPWLSATTVRSVTHANALT (also referred to herein as sequence $B3_{5A}$); SEQ ID NO:2: SVTHANALTVMGKASTPGAA (also referred to herein as sequence $B3_{5B}$); SEQ ID NO:3: GKASTPGAAAQIQEVKEQRI (also referred to herein as sequence $B3_{5C}$); SEQ ID NO:4:DRILLLFKPPKYHPDVPYVK (also referred to herein as sequence $B3_{6A}$); and unique fragments thereof, wherein the unique fragments (1) bind to an MSP-1 polypeptide and (2) exclude the sequences set forth in Table 4:Band 3 Blast Homology Sequences. According to another aspect of the invention, isolated nucleic acid molecules that encode the foregoing polypeptides are provided. According to yet another aspect of the invention, expression vectors that include the foregoing nucleic acid molecules operably linked to a promoter are provided. In another aspect of the invention host cells transfected or transformed with the expression vector are provided.

According to another aspect of the invention, immunogenic compositions are provided. The compositions include one or more of the foregoing isolated Band 3 polypeptides; and a pharmaceutically acceptable carrier; wherein the polypeptides are present in an effective amount to induce an immune system response. In some embodiments, the compositions also include an adjuvant. According to another aspect of the invention, methods of making a medicament are provided. The methods include placing one or more of the foregoing isolated Band 3 polypeptides in a pharmaceutically acceptable carrier.

According to yet another aspect of the invention, methods for identifying a candidate mimetic of the foregoing isolated Band 3 polypeptides are provided. The methods include providing an MSP-1 polypeptide which binds a foregoing Band 3 polypeptide, contacting the MSP-1 polypeptide with a test molecule, and determining the binding of the test molecule to the MSP-1 polypeptide, wherein a test molecule which binds to the MSP-1 polypeptide and inhibits binding of the MSP-1 polypeptide to the foregoing isolated Band 3 polypeptide is a candidate mimetic of the foregoing isolated Band 3 polypeptide.

According to another aspect of the invention, protein microarrays are provided. The microarrays include at least one isolated Band 3 polypeptide selected from the group consisting of SEQ ID NOS. 1, 2, 3, and 4.

According to yet another aspect of the invention, anti-Band 3 antibodies or fragments thereof are provided. The anti-Band 3 antibodies or fragments thereof selectively bind to a foregoing isolated Band 3 polypeptide, wherein the antibody inhibits infection of cells by *P. falciparum* merozoite malaria parasite. Preferably, the antibody is a monoclonal antibody, and, more preferably, a humanized monoclonal antibody.

According to another aspect of the invention, anti-idiotype antibodies which selectively bind to an idiotype of the foregoing Band 3 antibodies are provided. As used herein, an idiotype refers to a specific binding site of an antibody that binds to the peptide antigen. In accordance with the present invention, the anti-idiotype antibody blocks penetration of malaria parasite into human red blood cells, presumably by virtue of blocking the malarial parasite ligand that binds to the erythroid Band 3 protein receptor. According to a related aspect of the invention,

methods for making an anti-idiotypic antibody are provided. The methods include immunizing an animal with a foregoing Band 3 antibody under conditions to elicit an immune system response to an idiotype of said foregoing antibody.

According to some aspects of the invention, methods for treating a malaria infection, are provided. The methods include administering to a subject in need of such treatment, an effective amount of a foregoing anti-Band 3 antibody to treat the malaria infection. This method of treatment is referred to herein as "passive immunity".

According to another aspect of the invention, methods for inducing an immune system response to treat a malaria infection are provided. The methods include administering to a subject in need of such treatment, an effective amount of a foregoing anti-Band 3 antibody under conditions to induce an anti-idiotypic immune response to the anti-Band 3 antibody idiotype. This method of treatment is referred to herein as "active immunity".

The invention also is based, in part, on the discovery of the particular portion of MSP-1 (alternatively referred to herein as "MSP1") that selectively binds to Band 3 protein. Thus the invention embraces various compositions containing such MSP-1 peptides for use, e.g., in screening assays to detect the specific interaction between MSP-1 and Band 3 protein, as well as for use in diagnostic and therapeutic applications for detecting and treating, respectively, malaria infection. In general, such compositions contain components (or are contained in kits which contain additional components) which are selected to detect the specific interaction between MSP-1 and Band 3 protein (particularly the Band 3 polypeptides disclosed herein).

According to another aspect of the invention, methods for identifying a candidate mimetic of a MSP-1 polypeptide are provided. The methods include providing an isolated Band 3 polypeptide which binds a MSP-1 polypeptide, contacting the Band 3 polypeptide with a test molecule, and determining the binding of the test molecule to the Band 3 polypeptide, wherein a test molecule which binds to the isolated Band 3 polypeptide and inhibits binding of the Band 3 polypeptide to the MSP-1 polypeptide is a candidate mimetic of the MSP-1 polypeptide. In some embodiments, the MSP-1 polypeptide has a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:33, SEQ

ID NO. 34, and SEQ ID NO:35. In certain embodiments, the test molecule is an antibody.

According to another aspect of the invention, isolated polypeptides are provided. The polypeptides include an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 33, 34, and 35, or fragments thereof. According to another aspect of the invention, pharmaceutical compositions are provided. The compositions include one or more of the foregoing isolated MSP-1 polypeptides and a pharmaceutically acceptable carrier; wherein the polypeptides are present in an effective amount to induce an immune system response. In some embodiments, the pharmaceutical composition also includes an adjuvant. According to another aspect of the invention, methods of making a medicament are provided. The methods include placing one or more of the foregoing isolated MSP-1 polypeptides in a pharmaceutically acceptable carrier.

According to yet another aspect of the invention, methods of preventing or treating a malaria infection are provided. The methods include administering a foregoing MSP-1 pharmaceutical composition to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection. According to some aspects of the invention, malaria polypeptide binding polypeptides are provided. The malaria polypeptide binding polypeptides selectively binds to a foregoing isolated MSP-1 polypeptide. Preferably, the binding polypeptide is an antibody or antigen-binding fragment of an antibody. Preferably, the antibody is a monoclonal antibody, and more preferably, a humanized monoclonal antibody. According to another aspect of the invention, pharmaceutical compositions that include the foregoing malaria polypeptide binding polypeptide in a pharmaceutically acceptable carrier are provided.

According to another aspect of the invention, methods of preventing or treating a malaria infection are provided. The methods include administering the foregoing MSP-1 polypeptide binding polypeptide pharmaceutical composition to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection.

According to another aspect of the invention, isolated nucleic acids are provided. The isolated nucleic acids include a nucleotide sequence selected from the group consisting of SEQ ID NOs:54-59, or fragments thereof.

According to another aspect of the invention, an isolated Band 3 polypeptide is provided. The Band 3 polypeptide includes an amino acid sequence selected from the group consisting of SEQ ID NO. 1, 2, 3, and 4 as shown herein:

SEQ ID NO:1: GMPWLSATTVRSVTHANALT (also referred to herein as sequence $B3_{5A}$);

SEQ ID NO:2: SVTHANALTVMGKASTPGAA (also referred to herein as sequence $B3_{5B}$);

SEQ ID NO:3: GKASTPGAAAQIQEVKEQRI (also referred to herein as sequence $B3_{5C}$);

SEQ ID NO:4: DRILLLFKPPKYHPDVPYVK (also referred to herein as sequence $B3_{6A}$), and unique fragments thereof, wherein the unique fragments (1) bind to an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53, or fragment thereof, and (2) exclude the sequences set forth in Table 4:Band 3 Blast Homology Sequences.

According to another aspect of the invention, isolated nucleic acid molecules that encode the foregoing isolated Band 3 polypeptides are provided. According to another aspect of the invention, expression vectors are provided. The expression vectors include the foregoing isolated Band 3 nucleic acids operably linked to a promoter. According to another aspect of the invention, host cells transfected or transformed with the foregoing expression vectors are provided.

According to another aspect of the invention, immunogenic compositions are provided. The compositions include one or more of the foregoing isolated Band 3 polypeptides and a pharmaceutically acceptable carrier; wherein the Band 3 polypeptides are present in an effective amount to induce an immune system response. In some embodiments, the compositions also include an adjuvant. According to another aspect of the invention, methods of making a medicament are provided. The methods include placing one or more of the foregoing isolated Band 3 polypeptides in a pharmaceutically acceptable carrier.

According to another aspect of the invention, methods for identifying a candidate mimetic of a foregoing isolated Band 3 polypeptide are provided. The methods include providing a malaria polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53, or fragment thereof that binds the foregoing isolated Band 3 polypeptide or fragment thereof, contacting the malaria polypeptide or fragment thereof, with a test molecule, and determining the binding of the test molecule to the malaria polypeptide or fragment thereof, wherein a test molecule which binds to the polypeptide or fragment thereof and inhibits binding of the foregoing isolated Band 3 polypeptide to the malaria polypeptide, is a candidate mimetic of the foregoing isolated Band 3 polypeptide.

The invention also is based, in part, on the discovery of BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA polypeptides that selectively binds to Band 3 protein. Thus the invention embraces various compositions containing such BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA polypeptides for use, e.g., in screening assays to detect the specific interaction between the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA polypeptides and Band 3 protein, as well as for use in diagnostic and therapeutic applications for detecting and treating, respectively, malaria infection. In general, such compositions contain components (or are contained in kits which contain additional components) which are selected to detect the specific interaction between BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, or ABRA and Band 3 protein (particularly the Band 3 polypeptides disclosed herein).

According to another aspect of the invention, methods for identifying a candidate mimetic of an isolated malaria polypeptide are provided. The methods include providing a Band 3 molecule which binds a malaria polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53, contacting the Band 3 molecule with a test molecule, and determining the binding of the test molecule to the Band 3 molecule, wherein a test molecule which binds to the Band 3 molecule and inhibits binding of the malaria polypeptide with the Band 3 polypeptide is a candidate mimetic of the malaria polypeptide. In some embodiments, the test molecule is an antibody.

According to another aspect of the invention, isolated polypeptide molecules that include amino acid sequences selected from the group consisting SEQ ID NOs:46-52 are provided. According to another aspect of the invention, pharmaceutical compositions are provided. The pharmaceutical compositions include one or more of the foregoing isolated polypeptides and a pharmaceutically acceptable carrier; wherein the polypeptides are present in an effective amount to induce an immune system response. In some embodiments, the pharmaceutical composition also includes an adjuvant. According to another aspect of the invention, methods of making a medicament are provided. The methods include placing one or more of the foregoing isolated polypeptides in a pharmaceutically acceptable carrier. According to another aspect of the invention, methods of preventing or treating a malaria infection are provided. The methods include administering the foregoing pharmaceutical composition to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection.

According to another aspect of the invention, malaria polypeptide binding polypeptides are provided. The malaria polypeptide-binding polypeptides selectively bind to the foregoing isolated malaria polypeptides, e.g., the binding polypeptide is an antibody or antigen-binding fragment of an antibody. Preferably, the antibody is a monoclonal antibody, and more preferably, a humanized monoclonal antibody. According to another aspect of the invention, pharmaceutical compositions that include the foregoing malaria polypeptide binding polypeptide in a pharmaceutically acceptable carrier are provided.

According to another aspect of the invention, methods of preventing or treating a malaria infection are provided. The methods include administering the foregoing pharmaceutical composition to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection.

According to another aspect of the invention, isolated nucleic acid molecules are provided. The nucleic acid molecules are selected from the group consisting of:

(a) nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:38-44 and which codes for a *Plasmodium* polypeptide,

(b) deletions, additions and substitutions of the nucleic acid molecules of (a), which code for a *Plasmodium* polypeptide,

(c) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and

(d) complements of (a), (b) or (c).

In some embodiments, the isolated nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:38-44.

According to another aspect of the invention, isolated nucleic acid molecules are provided. The nucleic acid molecules are selected from the group consisting of:

(a) a unique fragment of the nucleotide sequence selected from the group consisting of:

nucleotides 1-1287 of SEQ ID NO:38 between 12 and 1286 nucleotides in length,
nucleotides 1-3576 of SEQ ID NO:39 between 12 and 3557 nucleotides in length,
nucleotides 1-903 of SEQ ID NO:40 between 12 and 902 nucleotides in length,
nucleotides 1-1203 of SEQ ID NO:41 between 12 and 1202 nucleotides in length,
nucleotides 1-3996 of SEQ ID NO:42 between 12 and 3995 nucleotides in length, and
nucleotides 1-876 of SEQ ID NO:43 between 12 and 875 nucleotides in length, and
nucleotides 1-2712 of SEQ ID NO:44 between 12 and 2711 nucleotides in length, and

(b) complements of (a),

wherein the unique fragments exclude nucleic acids having nucleotide sequences that are contained within SEQ ID NO:38-44, and that are known as of the filing date of this application.

According to another aspect of the invention, expression vectors that include the foregoing isolated nucleic acid molecules operably linked to a promoter are provided.

According to another aspect of the invention, isolated polypeptide molecules that include a unique fragment of amino acid sequence SEQ ID NO:53 that binds to a Band 3 polypeptide are provided.

These and other objects of the invention will be described in further detail in connection with the detailed description of the invention.

Brief Description of the Drawings

The figures are illustrative only and are not required for enablement of the inventions disclosed herein.

Figure 1 is a bar graph (Fig. 1A) depicting *P. falciparum* infection of mouse RBCs *in vitro* and a digitized image of a photomicrograph (Fig 1B) showing in Giemsa-stained thin smears at the end of the 24 h culture. The arrowhead indicates a newly infected RBC (ring stage parasite). The parasite culture was kept for 22-24 h at 37°C as described (Klotz, F.W., et al., *J Exp Med* 165:1713-1718, 1987). Rings were counted from 5,000 RBCs in Giemsa-stained thin smears. Data analyzed as the mean of triplicate experiments with standard error.

Figure 2 depicts the inhibition of *P. falciparum* invasion into RBCs by human Band 3 peptides. Fig. 2A shows a domain map illustration in (ii)-(iv), an inclusive boundary for each putative ectodomain was chosen as shown by vertical dotted lines. Ectodomains are shown as boxes in the three models. (i) Overlapping 12 to 20-residue peptides shown in solid bars were prepared according to the putative ectodomain boundaries. (ii) Casey model (Fujinaga, J., et al., J Biol Chem 274:6626-6633, 1999). (iii) Reithmeier model (Popov, M., et al., *J Biol Chem* 272:18325-18332, 1997). (iv) Sherman model (Crandall, I., et al., *Parasitology* 108:257-267, 1994). Fig. 2B shows a bar graph depicting an invasion inhibition assay by visual counting method, in which the number of ring stage parasites in 1,300-1,600 RBCs in Giemsa-stained thin smears was scored and plotted for each sample. Artemisinin (25 μM) and no peptide samples were positive and negative controls, respectively. Fig. 2C is a bar graph depicting of invasion inhibition assay by ^{3}H-hypoxanthine incorporation method. Effects of Band 3 peptides relative to the control sample (no peptide) are shown. Artemisinin (50 μM) and unrelated peptide derived from dematin were used as positive and negative control, respectively. Fig.2D is a bar graph that depicts results of a growth inhibition assay by ^{3}H-hypoxanthine incorporation method. Effects of Band 3 peptides relative to the control sample (no peptide) are shown. In all inhibition assays, mean parasitemia from three experiments was calculated with standard error. DMSO background was corrected when necessary. Student's *t* test

was used to compare with the control (no peptide). Fig. 2E is a table providing a summary of net charge and pI for Band 3 peptides.

Figure 3 shows results of binding of human Band 3 and native *P. falciparum* merozoite proteins. Fig. 3A is a digitized photomicrographic image of a Giemsa-stained smear depicting purified merozoites with malaria pigments but no contaminating RBC components. Fig. 3B is a digitized image of a merozoite protein separation. Total merozoite proteins from purified merozoites and human RBC ghost proteins were separated by SDS-PAGE (M, merozoite; G, RBC ghost). Residual human serum albumin (HSA; apparent mass 67 kDa) from the culture medium is marked with an asterisk in the Coomassie gel. Fig. 3C is a digitized image of a blot overlay assay. The 5C+6A mixture specifically bound to merozoite proteins (arrowheads) but not RBC proteins on the blot. Peptides 3A, 4A, and 2 as negative control showed non-specific bindings to HSA (asterisk) in M and α/β spectrin (240/220 kDa) in G. Fig. 3D is a digitized image of a gel depicting recombinant 5ABC and 5BC expressed in *E. coli*. Coomassie gel (lanes 1-3) and anti-GST Western blot (lanes 4-6) showed affinity purified GST-5BC (lanes 1, 4) and GST-5ABC (lanes 2, 5). GST control sample (lanes 3, 6). Fig. 3E is a digitized image demonstrating native MSP1 binding to 5ABC. Autoradiography showed radiolabeled MSP1 (full length) and $MSP1_{42}$ bound to GST-5ABC (lane 1) and mAb 5.2 (lane 3), but not to GST (lane 2). Results reproduced three times.

Figure 4 shows binding of recombinant human Band 3 and *P. falciparum* MSP1. Fig.4A is a digitized image of a Coomassie gel showing recombinant MSP1 and Band 3 (lanes 1-3) and a digitized image of an anti-GST Western blot (lanes 4-6) showing affinity purified GST-$MSP1_{42}$ (lanes 1, 4), truncated GST-$MSP1_{38}$ (lanes 2, 5), and GST as control (lanes 3, 6). Truncations of GST-$MSP1_{38}$ appear to be at the C-terminus as anti-GST antibody reacted with all three major Bands. GST-$MSP1_{19}$ was co-purified with GST (lane 7, Coomassie; lane 8, anti-GST blot; lane 9, anti-mAb 5.2 blot). Fig. 4A also depicts a digitized image of an autorad showing ^{32}P-labeled $MSP1_{19}$ (lane 10), 5BC (lane 11), and 5ABC (lane 12). Figs. 4B, 4C, and 4D show line graphs and a bar graph depicting results of a solution-binding assay. Binding

assays were performed as described in Example 5 and in Oh, S.S., et al., *Mol Biochem Parasitol* 108:237-247, 2000. ^{32}P-labeled 5ABC (10, 20, 40, 80 μM) and 5BC (21, 42, 84, 168 μM) respectively bound to GST-MSP1$_{42}$ (Fig. 4B) and GST-MSP1$_{38}$ (Fig. 4C) on beads in concentration-dependent manner. ^{32}P-labeled MSP1$_{19}$ bound specifically to the 5ABC domain (Fig. 4D) as statistically analyzed by Student's *t* test. Fig. 4E is a graphical summary of MSP1-Band 3 interactions. Dotted line denotes the C-terminal truncation of MSP1$_{38}$.

Figure 5. depicts results indicting *P. falciparum* MSP1 binds to intact RBCs in suspension. Fig. 5A is a digitized image of a 10% Coomassie gel of enzyme-treated RBCs (as described in Example 5). The gel (lanes 1-6) shows ghost membrane proteins prepared from untreated human (lane 1) and mouse (lane 4) RBCs, Nm-treated human (lane 2) and mouse (lane 5) RBCs, and ChT-treated (40 min) human (lane 3) and mouse (lane 6) RBCs. Arrowheads and arrows respectively indicate full-length and ChT-digested Band 3. The middle panel is a digitized image of a gel demonstrating PAS staining of the gel, which allowed analysis of sialic acid content in RBC ghosts prepared from untreated (lane 7), ChT-treated (lane 8), and Nm-treated (lane 9) human RBC samples. The right panel depicts a digitized image of a Western blot of ghost proteins prepared from untreated (lanes 10, 13), ChT-treated (lanes 11, 14), and Nm-treated (lane 12) human RBCs using anti-Band 3 and anti-GPA antibody are shown. Fig. 5B is a digitized image of an anti-GST Western blot showing Nm-treated (lane 1) and untreated (lane 3) human RBCs binding GST-MSP1$_{38}$. GST was used as control (lanes 2, 4). Fig. 5C is a digitized image depicting ^{32}P-labeled MSP1$_{19}$ binding to various RBC types. Assays were repeated 3-6 times. Means (± standard error) were plotted relative to the control (untreated wild-type RBCs) and compared using Student's *t* test.

Figure 6. is a digitized image of an invasion pathway model. In both sialic acid-independent and dependent pathways, Band 3 may function as an important RBC receptor for *P. falciparum* invasion. In the former pathway, Band 3 might be an independent receptor (open arrows) or complemented by GPA to take part in the sialic acid-dependent pathway (dotted arrow). In the latter pathway, GPA appears to be a

non-essential receptor perhaps requiring the coupling of the essential Band 3 receptor (solid black arrows).

Detailed Description of the Invention

For over two decades, the malaria parasite protein MSP-1 has been reported to play an important role during the parasite invasion of red blood cells based on the findings that (a) it is a major protein found on the surface of the merozoite, the invasive form of the malaria parasite, (b) full-length MSP-1, a segment of MSP1(38), as well as a number of 20-mer peptides derived from MSP-1 bound to erythrocytes, (c) MSP-1 induced antibodies provided protection against malaria infection in animal models, and (d) deleting a portion of the MSP-1 gene caused destruction of the parasite *in vitro*. However, the exact function of MSP-1 has remained unknown. We have identified for the first time a definitive function of MSP-1 in the malaria parasite invasion of red blood cells. This newly identified function revealed that specific proteolytic fragments of MSP-1 (i.e., MSP1(38), MSP1(42), MSP1(19)) are the parasite ligands specifically binding to the erythrocyte receptor Band 3 during invasion and that the binding interaction between the parasite MSP-1 fragments and the host Band 3 receptor is important for the invasion process to proceed successfully. We have identified that MSP1(19) – the 19 kDa C-terminal fragment of MSP-1 formed by secondary proteolytic processing of MSP1(42) – is also a parasite ligand binding to the Band 3 receptor peptides, e.g., SEQ ID NOs 1, 2, 3, and 4.

The functional form of MSP-1 during malaria parasite invasion into erythrocytes is not full-length MSP-1 (approximately 195-205 kDa depending upon the *Plasmodium falciparum* malaria strain) but its naturally processed proteolytic fragments generally known as MSP1(83), MSP1(30), MSP1(38), MSP1(42), and MSP1(33), and MSP1(19). The first four proteolytic fragments are formed upon primary processing of full-length MSP1. The last two are formed by secondary processing of MSP1(42). These primary and secondary processing products form a non-covalent complex on the surface of merozoites (the invasive form of the parasite) during invasion. However, only MSP1(19) which is anchored to the merozoite

membrane is carried into the newly infected erythrocyte while other fragments are shed into the surrounding medium.

In view of the foregoing, the invention provides isolated Band 3 peptides which selectively bind to merozoite surface protein-1 (MSP-1). The complete nucleic acid and amino acid sequences for human Band 3 protein are described in GenBank accession nos. X12609 and M27819 (SEQ ID NOs:5 and 6, (X12609 nucleic acid and amino acid sequences, respectively); and SEQ ID NOs:7 and 8 (M27819 nucleic acid and amino acid sequences, respectively); the complete amino acid and nucleic acid sequences for MSP-1 are described in GenBank accession no. X02919 (SEQ ID NOs:9 and 10, respectively). See also, (e.g., Fujinaga, J., et al. *J Biol Chem* 274:6626-6633, 1999) which reports the topology of the membrane domain of Band 3.

The results disclosed herein suggest that these particular Band 3 peptide sequences selectively interact with MSP-1 and, thereby, facilitate malaria parasite entry into erythrocytes. Such peptides are alternatively referred to herein as "Band 3 immunogenic polypeptides", "Band 3-derived MSP-1 binding peptides", and the like. Hence, one aspect of the invention is an isolated peptide selected from the group of sequences having SEQ ID Nos. 1, 2, 3, and 4, and unique fragments thereof which bind to MSP-1. The selection of these particular sequences and of the particular malaria protein with which they interact, could not have been predicted based on the information presently known regarding the Band 3 structure.

Although not wishing to be bound to any particular theory or mechanism, it is believed that *P. falciparum* depends on the expression on its surface of a specific molecule, MSP-1, also (referred to herein as a ligand), stereochemically complementary to the Band 3 receptor for its binding to and/or, subsequent penetration into the erythrocyte. This ligand can be inhibited in its binding to the receptor (in the specific case of *P. falciparum*, the receptor molecule corresponds to erythroid Band 3) by an antibody which selectively binds to SEQ ID NO. 1, 2, 3, and/or 4 (anti-Band 3 antibody) and which possesses the same sterochemical specificity. Thus, immunization with an anti-Band 3 antibody of the invention as a vaccine is useful for eliciting an immune response to its combining site, termed an anti-idiotypic response, resulting in antibodies which will also recognize the

immunologically identical epitope on the ligand and, hence, will protect the host against the erythrocytic forms (merozoites) of *P. falciparum*. Thus, the present invention also involves the use of monoclonal anti-Band 3 antibodies immunochemically specific for the epitope used by *P. falciparum* to penetrate into host cells. These monoclonal antibodies are used according to the present invention, to generate or elicit the corresponding anti-idiotypic antibodies which, by virtue of their specificity for the parasite's ligand molecule (MSP-1) are useful in the serodiagnosis and treatment of established infection.

As used herein a "subject" shall mean a human, vertebrate, or invertebrate animal including but not limited to a dog, cat, horse, cow, pig, sheep, goat, non-human primate (e.g. monkey), rabbit, rat, mouse, avian, or insect (e.g. a mosquito). The malarial parasites of the invention include: *P.falciparum, P.vivax, P.malariae, P.ovale, P.berghei, P.yoelii, P.chabaudi, P.vinckei, and P. knowlesi, P. cynomolgi, and P. coatneyi*. A preferred malarial parasite of the invention is *P.falciparum*. As used herein, a "malarial infection" includes infection with a malarial parasite including: *P.falciparum, P.vivax, P.malariae, P.ovale P.berghei, P.yoelii, P.chabaudi, P.vinckei, and P. knowlesi, P. cynomolgi, and/or P. coatneyi*. A preferred malaria infection of the invention is infection with *P.falciparum*.

As used herein, the term "cell" means a cell capable of being infected by, or suspected of being exposed to a malarial parasite. This may include cells in or from a subject and cells grown in culture. A cell may also mean a cell collected from a subject such as a human or animal, for example, blood collected for purposes such as, but not limited to, transfusions. In some embodiments, a cell may be a negative control cell, which may be a cell that has not been exposed to a *Plasmodium* parasite. In some embodiments, a positive control cell may be a cell that has been exposed to a *Plasmodium* parasite but is free of a pharmaceutical agent of the invention. A cell is any cell that can be infected by a *Plasmodium* parasite, which includes, but is not limited to: mammalian cells, human cells, avian cells, insect cells, arthropod cells, neuronal cells, ocular cells, erythrocytes, lymphocytes, muscle cells, and intestinal cells.

One class of subjects according to the present invention are subjects having a *Plasmodium* infection. Such subjects are subjects in need of treatment with a

Plasmodium inhibitor. This class of subjects includes subjects diagnosed with infection, exhibiting symptoms of infection, or having been exposed to a *Plasmodium* parasite. A subject at risk of developing a *plasmodium* infection is a subject in need of prevention of infection. Such subjects include those at risk of exposure to an infection-causing *Plasmodium* parasite. For instance, a subject at risk may be a subject who is planning to travel to an area where a particular type of infectious *Plasmodium* parasite is found or it may be a subject who through lifestyle, occupation, or medical procedures is exposed to bodily fluids which may contain a *Plasmodium* parasite or even any subject living in an area that a *Plasmodium* parasite has been identified. Subjects at risk of developing infection also include general populations to which a medical agency recommends preventative infectious measures for a particular infectious organism.

A subject may or may not exhibit symptoms of infection such as fever, swollen lymph glands, muscle aches, and pains. Methods to diagnose symptomatic and asymptomatic *Plasmodium* infection are known to those of ordinary skill in the medical arts and are described below herein. Some methods of diagnosis include, but are not limited to, blood tests for antibodies to the *Plasmodium* parasite and other assays such as lymph assays for *Plasmodium* parasites.

As noted above, the invention embraces functional variants, such as unique fragments, of the isolated Band 3 polypeptides of the invention which selectively bind to one or more of the peptides: MSP-1 (particularly SEQ ID NOS.:11,12,13, 33, 34, 35), and the polypeptide molecules comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53. As used herein, a "functional variant" or "variant" of a Band 3 polypeptide of the invention is a molecule which contains one or more modifications to the primary amino acid sequence of the Band 3 polypeptide of the invention and retains the MSP-1 binding properties disclosed herein. Modifications which create a Band 3 immunogenic polypeptide functional variant can be made, for example, 1) to enhance a property of a Band 3 binding peptide, such as peptide stability in an expression system or the stability of protein-protein binding such as MSP-1 binding; or 2) to provide a novel activity or property to a Band 3 immunogenic polypeptide, such as addition of an antigenic epitope or addition of a detectable moiety. Modifications to a Band 3 polypeptide of the invention can be

made to a nucleic acid which encodes the peptide, and can include deletions, point mutations, truncations, amino acid substitutions and additions of amino acids. Alternatively, modifications can be made directly to the polypeptide, such as by cleavage, addition of a linker molecule, addition of a detectable moiety, such as biotin, substitution of one amino acid for another and the like. Modifications also embrace fusion proteins comprising all or part of the Band 3 polypeptide amino acid sequence.

The amino acid sequence of Band 3 immunogenic polypeptides of the invention may be of natural or non-natural origin, that is, they may comprise a natural Band 3 polypeptide molecule or may comprise a modified sequence as long as the amino acid sequence retains the property of binding to MSP-1 and/or any of the other malaria polypeptides disclosed herein. For example, Band 3 polypeptides in this context may be fusion proteins of a Band 3 polypeptide of the invention and unrelated amino acid sequences, synthetic peptides of amino acid sequences shown in SEQ ID NOs:1, 2, 3, and 4, peptides isolated from cultured cells which express Band 3 peptides, and peptides coupled to nonpeptide molecules (for example in certain drug delivery systems or detectable labels).

Nonpeptide analogs of the Band 3 peptides of the invention, e.g., those which provide a stabilized structure or lessened biodegradation, are also contemplated. Peptide mimetic analogs can be prepared based on a selected Band 3 peptide by replacement of one or more residues by nonpeptide moieties. Preferably, the nonpeptide moieties permit the peptide to retain its natural confirmation, or stabilize a preferred, e.g., bioactive, confirmation. One example of a method for preparation of nonpeptide mimetic analogs from peptides is described in Nachman et al., *Regul. Pept.* 57:359-370 (1995). Peptide mimetics also can be selected from libraries of synthetic compounds (e.g. combinatorial libraries of small organic molecules) or natural molecules according to the MSP-1 and/or other malaria polypeptide binding properties of such molecule (i.e., ability to selectively bind to MSP-1 and/or other malaria polypeptides disclosed herein (isolated or expressed on the surface of a cell or organism)) and/or inhibit parasite entry into human red blood cells. In general, the methods for selection involve determining whether the library's molecules inhibit selective binding of a Band 3 peptide of the invention (e.g., SEQ ID NOs:1, 2, 3, or 4)

to MSP-1 and/or to other malaria polypeptides disclosed herein and/or block the malaria parasite invasion of red blood cells by for example inhibiting a natural process which merozoites use to penetrate red blood cells.

If a variant involves a change to an amino acid of a Band 3 polypeptide (e.g., SEQ ID NOs:1, 2, 3, or 4), functional variants of the Band 3 immunogenic polypeptide having conservative amino acid substitutions typically will be preferred, i.e., substitutions which retain a property of the original amino acid such as charge, hydrophobicity, conformation, etc. Examples of conservative substitutions of amino acids include substitutions made amongst amino acids within the following groups: (a) M, I, L, V; (b) F, Y, W; (c) K, R, H; (d) A, G; (e) S, T; (f) Q, N; and (g) E, D.

Other methods for identifying functional variants of the Band 3 polypeptides rely upon the development of amino acid sequence motifs to which potential epitopes may be compared. (See, e.g., published PCT application of Strominger and Wucherpfennig (US/96/03182)). In general, these methods rely upon the development of amino acid sequence motifs to which potential epitopes may be compared. Each motif describes a finite set of amino acid sequences in which the residues at each (relative) position may be (a) restricted to a single residue, (b) allowed to vary amongst a restricted set of residues, or (c) allowed to vary amongst all possible residues. For example, a motif might specify that the residue at a Band 3 peptide position may be any one of the residues valine, leucine, isoleucine, methionine, or phenylalanine; that the residue at the second position must be histidine; that the residue at the third position may be any amino acid residue; that the residue at the fourth position may be any one of the residues valine, leucine, isoleucine, methionine, phenylalanine, tyrosine or tryptophan; and that the residue at the fifth position must be lysine.

Sequence motifs for the Band 3 peptide functional variants can be developed by analysis of the MSP-1 (or other malaria polypeptides of the invention) contact points of the Band 3 polypeptides disclosed herein. By providing a detailed structural analysis of the residues involved in the binding of the malaria polypeptides of the invention to the Band 3 polypeptides disclosed herein, one of ordinary skill in the art is enabled to make predictions of sequence motifs for binding between such pairs of proteins.

Using these sequence motifs as search, evaluation, or design criteria, one of ordinary skill in the art is enabled to identify classes of peptides (functional variants of the Band 3 peptides disclosed herein) which have a reasonable likelihood of binding to and of interacting with MSP-1 (or other malaria polypeptides of the invention) to inhibit parasite entry into erythrocytes. These peptides can be synthesized and tested for activity as described herein. Use of these motifs, as opposed to pure sequence homology (which excludes many peptides which are antigenically similar but quite distinct in sequence) or sequence homology with unlimited "conservative" substitutions (which admits many peptides which differ at critical highly conserved sites), represents a method by which one of ordinary skill in the art can evaluate peptides for potential application in the treatment of disease, such as malaria infection.

The binding of the variant Band 3 peptides to MSP-1 (or other malaria polypeptides of the invention) then is determined according to standard procedures. For example, the variant peptide can be contacted with the MSP-1 which binds the Band 3 peptides of the invention (e.g., SEQ ID NOs:1, 2, 3, or 4) to form a complex of the variant peptide and MSP-1. This contacting can be performed in the presence of Band-3 expressed on erythrocytes to determine whether the variant peptide of Band 3 inhibits binding of the MSP-1 (e.g., expressed by a malaria parasite) and/or entry of the parasite into the erythrocyte.

Variant Band 3 peptides include "unique fragments" of the peptides having SEQ ID NOs:1, 2, 3, and 4. As used herein, a unique fragment is one that is a 'signature' for the larger nucleic acid. It, for example, is long enough to assure that its precise sequence is not found in molecules within the human genome outside of the Band 3 nucleic acids defined above (and human alleles). Those of ordinary skill in the art may apply no more than routine procedures to determine if a fragment is unique within the human genome. Unique fragments, however, exclude fragments completely composed of the nucleotide sequences of any of GenBank accession numbers identified in Table 4 or other previously published sequences as of the filing date of the priority documents for sequences listed in a respective priority document or the filing date of this application for sequences listed for the first time in this application which overlap the sequences of the invention. Thus, unique Band 3

peptide fragments exclude the previously reported peptides identified in Table 4 (Blast results for SEQ ID NOs:1, 2, 3, or 4).

Binding of the variant peptide to the MSP-1 (or other malaria polypeptides of the invention) and/or blocking of the entry of MSP-1 (or other malaria polypeptides of the invention) (e.g., expressed by malaria parasite, containing a detectable label) into erythrocytes (or other cells expressing Band 3) indicates that the variant peptide is a functional variant. The methods also can include the step of comparing the blocking of Band 3-mediated MSP-1 (or other malaria polypeptides of the invention) (e.g., expressed on the merozoite surface) entry into erythrocytes by the Band 3 peptides or antibodies thereto (anti-Band 3 antibodies), as well as anti-idiotype antibodies, and the blocking by the functional variant as a determination of the effectiveness of the blocking by the functional variant. By comparing the functional variant with the Band 3 peptides or other compositions of the invention disclosed herein, peptides with increased Band 3 blocking properties can be prepared.

Variants of the Band 3 peptides prepared by any of the foregoing methods can be sequenced, if necessary, to determine the amino acid sequence and thus deduce the nucleotide sequence which encodes such variants. Thus, those nucleic acid sequences which code for a Band 3 peptide or variants thereof, including allelic variants, are also a part of the invention. In screening for nucleic acids which encode a Band 3 peptide of the invention, nucleic acid hybridization such as a Southern blot or a Northern blot may be performed under stringent conditions, together with a ^{32}P probe. The term "stringent conditions" as used herein refers to parameters with which the art is familiar. Nucleic acid hybridization parameters may be found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. Exemplary stringent conditions include hybridization at 65°C in hybridization buffer (3.5 x SSC, 0.02% Ficoll, 0.02% Polyvinyl pyrolidone, 0.02% Bovine Serum Albumin, 25mM NaH_2PO_4 (pH7), 0.5% SDS, 2mM EDTA). SSC is 0.15M Sodium Chloride/0.015M Sodium Citrate, pH 7; SDS is Sodium Dodecyl Sulphate; and EDTA is Ethylene diaminetetraacetic acid. After hybridization, the membrane upon which the DNA is transferred can be washed,

for example, at 2xSSC at room temperature and then at 0.1 - 0.5x SSC/0.1 x SDS at temperatures up to 68°C. After washing the membrane to which DNA encoding a Band 3 polypeptide is finally transferred, the membrane can be placed against X-ray film to detect the radioactive signal.

There are other conditions, reagents, and so forth which can be used, which result in a similar degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of nucleic acids encoding the Band 3 peptides of the invention. The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

The invention also includes the use of nucleic acid sequences which include alternative codons that encode the same amino acid residues of the Band 3 peptides of the invention. For example, leucine residues can be encoded by the codons CUA, CUC, CUG, CUU, UUA and UUG. Each of the six codons is equivalent for the purposes of encoding a leucine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the leucine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, *in vitro* or *in vivo*, to incorporate a leucine residue. Similarly, nucleotide sequence triplets which encode other amino acid residues comprising the Band 3 peptides include: GUA, GUC, GUG and GUU (valine codons); GGU, GGA, GGG, GGC (glycine codons); UAC and UAU (tyrosine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the native Band 3 peptide encoding nucleic acids in codon sequence due to the degeneracy of the genetic code.

Preferred nucleic acids encoding Band 3 polypeptides are those which preferentially express Band 3 peptides, such as those having SEQ ID NOs:1, 2, 3, or 4. The Band 3 nucleic acids of the invention do not encode the entire Band 3 polypeptide but do include nucleotide sequences encoding the Band 3 peptides disclosed herein.

The invention also provides modified nucleic acid molecules which include additions, substitutions and deletions of one or more nucleotides. In preferred embodiments, these modified nucleic acid molecules and/or the polypeptides they encode retain at least one activity or function of the unmodified nucleic acid molecule and/or the polypeptides, such as antigenicity, ligand binding, formation of complexes by binding of peptides to MSP-1 (or other malaria polypeptides of the invention), etc. In certain embodiments, the modified nucleic acid molecules encode modified polypeptides, preferably polypeptides having conservative amino acid substitutions as are described elsewhere herein. The modified nucleic acid molecules are structurally related to the unmodified nucleic acid molecules and in preferred embodiments are sufficiently structurally related to the unmodified nucleic acid molecules so that the modified and unmodified nucleic acid molecules hybridize under stringent conditions known to one of skill in the art.

For example, modified nucleic acid molecules which encode polypeptides having single amino acid changes can be prepared. Each of these nucleic acid molecules can have one, two or three nucleotide substitutions exclusive of nucleotide changes corresponding to the degeneracy of the genetic code as described herein. Likewise, modified nucleic acid molecules which encode polypeptides having two amino acid changes can be prepared which have, e.g., 2-6 nucleotide changes. Numerous modified nucleic acid molecules like these will be readily envisioned by one of skill in the art, including for example, substitutions of nucleotides in codons encoding amino acids 2 and 3, 2 and 4, 2 and 5, 2 and 6, and so on. In the foregoing example, each combination of two amino acids is included in the set of modified nucleic acid molecules, as well as all nucleotide substitutions which code for the amino acid substitutions. Additional nucleic acid molecules that encode polypeptides having additional substitutions (i.e., 3 or more), additions or deletions (e.g., by introduction of a stop codon or a splice site(s)) also can be prepared and are embraced by the invention as readily envisioned by one of ordinary skill in the art. Any of the foregoing nucleic acids or polypeptides can be tested by routine experimentation for retention of structural relation or activity to the nucleic acids and/or polypeptides disclosed herein.

It will also be understood that the invention embraces the use of the sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g., *E. coli*), or eukaryotic (e.g., CHO cells, COS cells, yeast expression systems and recombinant baculovirus expression in insect cells). The expression vectors require that the pertinent sequence, i.e., those described *supra*, be operably linked to a promoter.

Delivery of expression vectors encoding the Band 3 sequences *in vivo* and/or *in vitro* can be via the use of nucleic acid delivery systems known in the art (see, e.g., Allsopp et al., *Eur. J. Immunol.* 26(8):1951-1959, 1996). Recombinant vectors including viruses selected from the group consisting of adenoviruses, adeno-associated viruses, poxviruses including vaccinia viruses and attenuated poxviruses such as NYVAC, Semliki Forest virus, Venezuelan equine encephalitis virus, retroviruses, Sindbis virus, and Ty virus-like particle, plasmids (e.g. "naked" DNA), bacteria (e.g. the bacterium Bacille Calmette Guerin, BCG), and the like can be used in such delivery, for example, for use as a vaccine. Other viruses, expression vectors and the like which are useful in preparation of a vaccine are known to one of ordinary skill in the art. One can test the Band 3 delivery systems in standard model systems such as mice to determine efficacy of the delivery system. The systems also can be tested in human clinical trials.

As used herein, a "vector" may be any of a number of nucleic acids into which a desired sequence may be inserted by restriction and ligation for transport between different genetic environments or for expression in a host cell. Vectors are typically composed of DNA although RNA vectors are also available. Vectors include, but are not limited to, plasmids, phagemids, bacteria and virus genomes as disclosed herein, such as adenovirus, poxvirus and BCG. A cloning vector is one which is able to replicate in a host cell or be replicated after its integration into the genome of a host cell, and which is further characterized by one or more endonuclease restriction sites at which the vector may be cut in a determinable fashion and into which a desired DNA sequence may be ligated such that the new recombinant vector retains its ability to replicate in the host cell. In the case of plasmids, replication of the desired sequence may occur many times as the plasmid increases in copy number within the host bacterium or just a single time per host before the host reproduces by mitosis. In

the case of phage, replication may occur actively during a lytic phase or passively during a lysogenic phase. An expression vector is one into which a desired DNA sequence may be inserted by restriction and ligation such that it is operably joined to regulatory sequences and may be expressed as an RNA transcript. Vectors may further contain one or more marker sequences suitable for use in the identification of cells which have or have not been transformed or transfected with the vector. Markers include, for example, genes encoding proteins which increase or decrease either resistance or sensitivity to antibiotics or other compounds, genes which encode enzymes whose activities are detectable by standard assays known in the art (e.g., ß-galactosidase, luciferase or alkaline phosphatase), and genes which visibly affect the phenotype of transformed or transfected cells, hosts, colonies or plaques (e.g., green fluorescent protein). Preferred vectors are those capable of autonomous replication and expression of the structural gene products present in the DNA segments to which they are operably joined.

As used herein, a coding sequence and regulatory sequences are said to be "operably" joined when they are covalently linked in such a way as to place the expression or transcription of the coding sequence under the influence or control of the regulatory sequences. If it is desired that the coding sequences be translated into a functional protein, two DNA sequences are said to be operably joined if induction of a promoter in the 5' regulatory sequences results in the transcription of the coding sequence and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter region to direct the transcription of the coding sequences, or (3) interfere with the ability of the corresponding RNA transcript to be translated into a protein. Thus, a promoter region would be operably joined to a coding sequence if the promoter region were capable of effecting transcription of that DNA sequence such that the resulting transcript might be translated into the desired protein or polypeptide. As noted above, certain preferred nucleic acids express only fragments of Band 3 polypeptides which bind to the malaria peptides disclosed herein.

The precise nature of the regulatory sequences needed for gene expression may vary between species or cell types, but shall in general include, as necessary, 5' non-transcribed and 5' non-translated sequences involved with the initiation of

transcription and translation respectively, such as a TATA box, capping sequence, CAAT sequence, and the like. Especially, such 5' non-transcribed regulatory sequences will include a promoter region which includes a promoter sequence for transcriptional control of the operably joined gene. Regulatory sequences may also include enhancer sequences or upstream activator sequences as desired. The vectors of the invention may optionally include 5' leader or signal sequences. The choice and design of an appropriate vector is within the ability and discretion of one of ordinary skill in the art.

Expression vectors containing all the necessary elements for expression are commercially available and known to those skilled in the art. See, e.g., Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, 1989. Cells are genetically engineered by the introduction into the cells of heterologous DNA (RNA) encoding a Band 3 peptide of the invention. That heterologous DNA (RNA) is placed under operable control of transcriptional elements to permit the expression of the heterologous DNA in the host cell.

Preferred systems for mRNA expression in mammalian cells are those such as pcDNA3.1 (available from Invitrogen, Carlsbad, CA) that contain a selectable marker such as a gene that confers G418 resistance (which facilitates the selection of stably transfected cell lines) and the human cytomegalovirus (CMV) enhancer-promoter sequences. Additionally, suitable for expression in primate or canine cell lines is the pCEP4 vector (Invitrogen), which contains an Epstein Barr virus (EBV) origin of replication, facilitating the maintenance of plasmid as a multicopy extrachromosomal element. Another expression vector is the pEF-BOS plasmid containing the promoter of polypeptide Elongation Factor 1α, which stimulates efficiently transcription *in vitro*. The plasmid is described by Mishizuma and Nagata (*Nuc. Acids Res.* 18:5322, 1990), and its use in transfection experiments is disclosed by, for example, Demoulin (*Mol. Cell. Biol.* 16:4710-4716, 1996). Still another preferred expression vector is an adenovirus, described by Stratford-Perricaudet, which is defective for E1 and E3 proteins (*J. Clin. Invest.* 90:626-630, 1992). The use of the adenovirus to express proteins for immunization is disclosed by Warnier et al., in intradermal injection in mice for immunization against P1A (*Int. J. Cancer*, 67:303-310, 1996).

The invention also embraces so-called expression kits, which allow the artisan to prepare a desired expression vector or vectors. Such expression kits include at least separate portions of at least two of the previously discussed materials. Other components may be added, as desired.

The invention further includes nucleic acid or protein microarrays which include Band 3 nucleic acids or peptides of the invention (preferably at least one isolated Band 3 peptide selected from the group consisting of SEQ ID NO:1, 2, 3, or 4) or nucleic acids encoding such peptides. In this aspect of the invention, standard techniques of microarray technology are utilized to assess expression of the Band 3 binding peptides (e.g., anti-Band 3 antibodies) and/or identify biological constituents that bind such peptides. The constituents of biological samples include antibodies, MSP-1 molecules, other of the malaria peptide disclosed herein, and the like. Microarray technology, which is also known by other names including: protein chip technology and solid-phase protein array technology, is well known to those of ordinary skill in the art and is based on, but not limited to, obtaining an array of identified peptides or proteins on a fixed substrate, binding target molecules or biological constituents to the peptides, and evaluating such binding. See, e.g., G. MacBeath and S.L. Schreiber, "Printing Proteins as Microarrays for High-Throughput Function Determination," *Science* 289(5485):1760-1763, 2000.

Microarray substrates include but are not limited to glass, silica, aluminosilicates, borosilicates, metal oxides such as alumina and nickel oxide, various clays, nitrocellulose, or nylon. The microarray substrates may be coated with a compound to enhance synthesis of a probe (peptide or nucleic acid) on the substrate. Coupling agents or groups on the substrate can be used to covalently link the first nucleotide or amino acid to the substrate. A variety of coupling agents or groups are known to those of skill in the art. Peptide or nucleic acid probes thus can be synthesized directly on the substrate in a predetermined grid. Alternatively, peptide or nucleic acid probes can be spotted on the substrate, and in such cases the substrate may be coated with a compound to enhance binding of the probe to the substrate. In these embodiments, presynthesized probes are applied to the substrate in a precise, predetermined volume and grid pattern, preferably utilizing a computer-controlled robot to apply probe to the substrate in a contact-printing manner or in a non-contact

manner such as ink jet or piezo-electric delivery. Probes may be covalently linked to the substrate.

In some embodiments, one or more control peptide or nucleic acid molecules are attached to the substrate. Preferably, control nucleic acid molecules allow determination of factors such as binding characteristics, reagent quality and effectiveness, hybridization success, and analysis thresholds and success.

The present invention also concerns a general method for producing immunity against an infectious microorganism based on the stereochemical complementarity between the ligand molecule on the microorganism and its receptor in the host's cell. This complementarity is used according to the present invention to generate antibodies against the ligand by immunizing with monoclonal antibodies against the ligand-binding region of the receptor molecule. Such monoclonal anti-receptor antibodies, which react with the exact area of binding of the ligand molecule of the parasitic organism, bring about protective immunity because they elicit anti-idiotypic antibodies that react with the stereochemically equivalent region of the ligand.

The anti-Band 3 antibodies of the inventions selectively bind to SEQ ID NOs:1, 2, 3, and/or 4 and, thereby block penetration of *P. falciparum* malaria parasite into human red blood cells by virtue of effectively blocking the site on the erythrocytic molecule (Band 3) used as a target by the *P. falciparum* malaria parasite. Accordingly, the invention provides monoclonal antibodies which have a combining site that has the same stereochemical configuration as the ligand site (e.g., MSP-1) on the *P. falciparum* malaria parasite. Such anti-Band 3 antibodies and anti-idiotpyic antibodies are prepared by standard methods. The selected cloned hybridomas produce as large quantities of suitable monoclonal antibodies as desired.

In view of the foregoing, the invention also permits the artisan to treat a subject having a malaria infection. Treatments include administering an anti-Band 3 binding peptide (e.g., an anti-Band 3 antibody) or other agent which inhibits binding of MSP-1 (or other malaria polypeptides of the invention) expressed by a malaria parasite to Band 3 expressed by an erythrocyte. Agents useful in the foregoing treatments include Band 3 polypeptides and functional variants thereof, as well as the anti-Band 3 antibodies and anti-idiotypic antibodies of the invention disclosed herein.

The function or status of a pharmaceutical agent as an *Plasmodium* inhibitor, can be determined according to assays known in the art or described herein. For example, cells can be contacted with a putative pharmaceutical agent and a *Plasmodium* parasite, and standard procedures can be used to determine whether the parasite is inhibited in its ability to enter or infect the cells. Such methods may also be utilized to determine the status of analogs, variants, derivatives, and fragments as inhibitors of invasion by *Plasmodium* parasites. One method for inhibiting infection is by inhibiting entry of *Plasmodium* parasite into cells. The ability to inhibit entry of *Plasmodium* parasite into cells with a putative pharmaceutical agent can be assessed using routine screening assays, e.g. by determining the level of entry of *Plasmodium* parasite into cells with and without the presence of the putative pharmaceutical agent.

Once the pharmaceutical agents are verified as modulating *Plasmodium* parasitic infection using secondary assays as described above herein, further biochemical and molecular techniques may be used to identify the targets of these compounds and to elucidate the specific roles that these target molecules play in the process of invasion. An example, though not intended to be limiting, is that the compound(s) may be labeled and contacted with a parasite to identify the host cell proteins with which these compounds interact. Such proteins may be purified, e.g., by labeling the compound with an immunoaffinity tag and applying the protein-bound compound to an immunoaffinity column.

In addition, the status of a pharmaceutical agent as a *Plasmodium* parasite toxin can be identified by using methods provided herein to determine the presence of a functional, active *Plasmodium* parasite. The agent may for example be assayed in the context of a material, for example a water sample, before and after contact with the sample and the pharmaceutical agent.

When administered, the therapeutic compositions of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents.

The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. The characteristics of the carrier will depend on the route of administration.

The therapeutics of the invention can be administered by any conventional route, including injection or by gradual infusion over time. The administration may, for example, be oral, intravenous, intraperitoneal, intramuscular, intranasal, intracavity, subcutaneous, intradermal, or transdermal.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

The preparations of the invention are administered in effective amounts. An effective amount is that amount of a pharmaceutical preparation that alone, or together with further doses, stimulates the desired response. In the case of treating an infectious disease such as a *Plasmodium* infection, the desired response is inhibiting the onset, stage or progression of the disease or infection. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. An effective amount for preventing infection is that amount that reduces the incidence of active infection when the cell or subject is exposed to the parasite, with respect to that amount that would occur in the absence of the active agent.

In another aspect of the invention, cell models and/or non-human animal models of *Plasmodium* infection may be produced by administering a molecule of the invention, such as an antibody, that inhibits *Plasmodium* infection. In some cases, a molecule of the invention that enhances *Plasmodium* invasion may be administered to an animal or cell. Such models may be useful for testing treatment strategies, monitoring clinical features of disease, or as tools to assess prevention strategies of *Plasmodium* infection. Cells and animal models made using enhancing molecules of

the invention may also be useful for assessing the ability of lead compounds to inhibit *Plasmodium* infection. For example, a cell contacted with an enhancer of invasion of the invention may be further contacted with putative agents that are candidate or lead compounds for treating or preventing *Plasmodium* infection. The ability of the lead or candidate compound to prevent or treat the infection may be evaluated in the model cell or animal. In addition the enhancers may serve as valuable lead compounds in that if their targets (by definition functionally important) can be identified and characterized, it may subsequently be possible to rationally design new compounds that act as inhibitors of these targets. As used herein, an "effective amount of an enhancer" is that amount effective to enhance *Plasmodium* parasitic infection. Such enhancements can be determined using standard assays as described above herein. Measurements of *Plasmodium* parasitic infection, are known to those of ordinary skill in the art and may vary depending on the specific parasite.

The pharmaceutical compound dosage may be adjusted by the individual physician or veterinarian, particularly in the event of any complication. A therapeutically effective amount typically varies from 0.01 mg/kg to about 1000 mg/kg, preferably from about 0.1 mg/kg to about 200 mg/kg, and most preferably from about 0.2 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or more days.

The absolute amount will depend upon a variety of factors, including the material selected for administration, whether the administration is in single or multiple doses, and individual patient parameters including age, physical condition, size, weight, and the stage of the disease. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

Diagnostic tests known to those of ordinary skill in the art may be used to assess *Plasmodium* infection status of a subject and to evaluate a therapeutically effective amount of a pharmaceutical agent administered. Examples of diagnostic tests are set forth below. A first determination of *Plasmodium* infection may be obtained using one of the methods described below (or other methods known in the art), and a subsequent determination of infection may be done. A comparison of the infection levels may be used to assess the effectiveness of administration of a

pharmaceutical agent of the invention as a prophylactic or a treatment of the *Plasmodium* infection. Absence of a *Plasmodium* infection may be an indication for prophylactic intervention by administering a pharmaceutical agent described herein to prevent *Plasmodium* infection.

Tests useful for diagnosis of *Plasmodium* infections are known to those of ordinary skill in the art. For example, diagnosis of malaria can be done by microscopic identification of asexual forms of the parasite in peripheral blood smears stained with Romanovsky staining, or Giemsa at pH 7.2, Wright's, Field's, or Leishman's stain. Both thin and thick blood smears may be examined. In addition, a finger-prick blood test is also available, in which the presence of *P. falciparum* histidine-rich protein 2 is determined. Additional methods of diagnosis and assessment of *Plasmodium* infection are known to those of skill in the art. The level of parasitemia may be important in the prognosis and can be determined with the above-identified diagnostic tests and by other means known in the art.

In addition to the diagnostic tests described above, clinical features of *Plasmodium* infection can be monitored for assessment of infection. These features include, but are not limited to: normochromic, nomocytic anemia, erythrocyte sedimentation rate, plasma viscosity, and platelet count may be reduced. Subjects may also have metabolic acidosis, with low plasma concentrations of glucose, sodium, bicarbonate, calcium, phosphate, and albumin together with elevations in lactate, blood urea nitrogen, creatinine, urate, muscle and liver enzymes, and conjugated and unconjugated bilirubin. In adults and children with cerebral malaria, the mean opening pressure at lumbar puncture is about 160 mm cerebrospinal fluid; the cerebrospinal fluid usually is normal or has a slightly elevated total protein level [<1.0 g/L (100 mg/dL)] (see Harrison's Principles of Internal Medicine, 14/e, McGraw Hill Companies, New York, 1998).

The identification of *Plasmodium* parasites in or on an object, may be performed via standard diagnostic methods described above including microscopic examination, antibody labeling in a sample of the object, and by PCR analysis of a sample.

The pharmaceutical agents of the invention may be administered alone, in combination with each other, and/or in combination with other anti-*Plasmodium* drug

therapies. Anti-malarial agents (for treatment and/or prophylaxis) that may be administered with pharmaceutical agents of the invention include, but are not limited to: mefloquine, doxycycline, chloroquine, aminoquinolines, dihydrofolate reductase inhibitors: pyrimethamine and proguanil (chloroguanide), dapsone, quinidine gluconate, quinine, artemisinin derivatives: artemether and artesunate, and primaquin. Methods of anti-*plasmodium* treatment of the invention may also be used in combination with drugs that target sialic acid interactions.

The above-described drug therapies are known to those of ordinary skill in the art and are administered by modes known to those of skill in the art. The drug therapies are administered in amounts that are effective to achieve the physiological goals (to reduce *Plasmodium* infection, and/or reduce *Plasmodium* parasite titer in a subject), in combination with the pharmaceutical agents of the invention. Thus, it is contemplated that the drug therapies may be administered in amounts which are not capable of preventing or reducing the physiological consequences of the *Plasmodium* infections when the drug therapies are administered alone, but which are capable of preventing or reducing the physiological consequences of *Plasmodium* infection when administered in combination with the pharmaceutical agents of the invention.

The pharmaceutical agents of the invention may also be administered in conjunction with vaccine formulations administered to confer immunity to a subject at risk of exposure to *Plasmodium* infection, which thereby prevents, reduces the severity of, or delays the onset of a subsequent *Plasmodium* infection.

The invention also provides a pharmaceutical kit comprising one or more containers comprising one or more of the pharmaceutical agents of the invention and or formulations of the invention. The kit may also include instructions for the use of the one or more pharmaceutical agents or formulations of the invention for the treatment of *Plasmodium* infection.

In other aspects the invention involves preventing and/or treating *Plasmodium* contamination of materials. A "material" as used herein is any liquid or solid material including, but not limited to: blood, tissue, bodily fluids, and tissue-processing equipment, including but not limited to: equipment for food processing, medical equipment, equipment for tissue transplant processing, and equipment for cell or bodily fluid processing. In some embodiments of the invention, the material is

aqueous. In some embodiments, the material is water, an example of which, although not intended to be limiting, is drinking water. The invention also involves preventing and/or treating *Plasmodium* contamination in blood, bodily fluids, cells, and tissue samples, including those from live human subjects and cadavers, as well as live animals and animal tissues and cells processed as food, cosmetics, or medication. As used herein, the term "contamination" means contact between the material and a *Plasmodium* parasite.

The isolated Band 3 peptides or anti-Band 3 antibodies of the invention may be combined with materials such as adjuvants to produce vaccines to prepare, respectively, anti-Band 3 antibodies that selectively bind to the portion of Band 3 involved in binding to MSP-1 and to prepare anti-idiotype antibodies that selectively bind to the portion of MSP-1 involved in binding to Band 3. Vaccines also encompass expression vectors and naked DNA or RNA, encoding a Band 3 peptide or anti-Band 3 antibody of the invention, precursors thereof, or fusion proteins thereof, which may be produced *in vitro* and administered via injection, particle bombardment, nasal aspiration and other methods. Vaccines of the "naked nucleic acid" type have been demonstrated to provoke an immunological response including generation of CTLs specific for the peptide encoded by the naked nucleic acid (*Science* 259:1745-1748, 1993).

In certain embodiments, the Band 3 peptides and anti-Band 3 antibodies of the invention are used to produce antibodies ("anti-Band 3 antibodies") which, in turn, may be used to produce "anti-idiotypic Band 3 antibodies", using standard techniques well known to the art. Standard reference works setting forth the general principles of antibody production include Catty, D., Antibodies, A Practical Approach, Vol. 1, IRL Press, Washington DC (1988); Klein, J., Immunology: The Science of Cell-Non-Cell Discrimination, John Wiley and Sons, New York (1982); Kennett, R., et al., Monoclonal Antibodies, Hybridoma, A New Dimension In Biological Analyses, Plenum Press, New York (1980); Campbell, A., Monoclonal Antibody Technology, in Laboratory Techniques and Biochemistry and Molecular Biology, Vol. 13 (Burdon, R. et al. EDS.), Elsevier Amsterdam (1984); and Eisen, H.N., Microbiology, third edition, Davis, B.D. et al. EDS. (Harper & Rowe, Philadelphia (1980). See also, U.S. patent no. 5,101,017, issued March 31, 1992 to Rubinstein, et al., entitled,

"Antibodies for providing protein against P. vivax malaria infection," which also reports the preparation of anti-idiotypic antibodies for treating infectious disease.

References which report vaccine approaches for treating malaria include: U.S. patent no. 6,066,623, issued to Hoffman, et al., entitled "Polynucleotide vaccine protective against malaria, methods of protection and vector for delivering polynucleotide vaccines"; and U.S. patent no. 6,120,770, issued to Adams et al., entitled "*Plasmodium* proteins useful for preparing vaccine compositions." Additional references which report anti-idiotype vaccines for treating various disorders include: Bendandi, *Leukemia,* 2000, 14(8):1333-9; Bhattacharya-Chatterjee et al., *Immunol. Lett.,* 2000,15:74(1):51-8; Maruyama et al., *Cancer Immunol. Immunother.,* 2000, 49(3):123-32; Herlyn et al., *Exp. Clin. Immunogenet.*, 1988, 5(4):165-75; Finberg et al., *Crit. Rev. Immunol.* 1987, 7(4):269-84; Nisonoff, *American Association of Immunologists,* 1991, 147(8):2429-2438; Greenspan et al., *FASEB J.,* 1993, 7:437-444; and Syrengelas et al., *The Journal of Immunology,* 1999, 162:4790-4795.

Thus, according to one aspect of the invention, an anti-Band 3 antibody (or fragment thereof) that selectively binds to a peptide having SEQ ID NO:1, 2, 3, and/or 4 and which blocks penetration of *P. falciparum* merozoite malaria parasite into human red blood cells is provided. According to yet another aspect of the invention, an anti-idiotype antibody which selectively binds to the idiotype of the anti-Band 3 antibodies described herein is provided. The antibodies of the present invention are prepared using any of a variety of methods, including administering the Band 3 peptides of the invention, fragments of the foregoing, antibodies selective for the foregoing, and the like to an animal to induce monoclonal or polyclonal antibodies. The production of monoclonal antibodies is according to techniques well known in the art.

Since the antibodies selective for the Band 3 epitope(s) (herein, anti-Band 3 antibodies) and the malaria polypeptide ligand(s) on the parasite recognize the same epitope, antibodies specific for the combining site on the anti-Band 3 antibodies (anti-idiotypic antibodies), can be elicited, which will react with the parasite's ligand(s). Susceptible individuals who make these anti-idiotypic antibodies will be protected against *P. falciparum* merozoites because (a) they block the ability of the parasite to

recognize the erythrocytic receptor and (b) they may induce the lysis or inactivation of the *Plasmodium* cell by fixing complement.

The monoclonal anti-Band 3 antibodies according to the invention have the following characteristics and properties:

(1) The antibodies can be used as the immunogenic agent in a vaccine and can be produced in virtually limitless quantity.

(2) The region of the antibody molecule that bears the immunogenic moiety (idiotype) is located in the variable region which may be further purified in order to avoid the undesired immunogenicity of the constant region of the molecule.

(3) If desired, the antigen combining region of the antibody can be transferred to a carrier molecule devoid of additional immunogenic properties for human subjects. As discussed below, this may be done by a number of methods that are equivalent in this regard, such as, by chemically binding the Fab fragment of the antibody molecule to an Fc fragment derived from human immunoglobulin or by genetically engineering an appropriate hybrid molecule using the necessary portion of the rearranged immunoglobulin heavy and light chain genes from the monoclonal-producing hybridoma cell line into human immunoglobulin genes from which the equivalent regions have been excised. Alternatively, the idiotype-bearing portions of the protein (or the DNA fragments encoding them) may be attached to other immunogenic molecules or particles (or to their respective genetic determinants in the case of the DNA fragments encoding the idiotypes).

(4) The Band 3 peptides of the invention may be used for binding to and isolating the parasite's specific recognition molecule(s) (e.g., MSP-1). The purified ligand molecule(s) from the merozoite can then be characterized and used as "blue-prints" for the preparation of synthetic peptides (Band 3 functional variants) with protective immunogenic properties.

(5) The antibodies can be used to prepare anti-idiotypic monoclonal antibodies in mice. Those anti-idiotypic antibodies that additionally react with the combining site of *P. falciparum* merozoites can be used as affinity probes, to isolate the ligand as is described in (4) above for the receptor on red blood cells and with the same objectives.

(6) The antibodies and the anti-idiotypic antibody can be used in the immunodiagnosis of *P. falciparum* infection. Thus, the presence of *P. falciparum* antigen in serum or other fluid may be detected and its concentration measured by its interference with the binding of the monoclonal anti-Band 3 to either the Band 3 molecule or to its monoclonal anti-idiotypic antibody. Since the parasite's ligand(s) and the anti-Band 3 antibodies will react with the same respective combining site both on the Band 3 molecule and in the monoclonal anti-idiotypic immunoglobulin, a simple competition assay can be designed using either enzyme-linked or radiolabeled reagents, or other labeling reagents.

(7) The anti-Band 3 antibodies may be used directly *in vivo* to block the red cell receptors for the parasite. This might be useful in the management of patients with particularly severe attacks of *P. falciparum* malaria, in whom the level of parasitemia may be very high. In the same type of patients, but not simultaneously, passively administered anti-idiotypic antibodies may be useful by directly binding to and destroying the parasites.

The present invention also provides a method for the detection of the presence of *P. falciparum* infection in a patient. The method employs insolubilized monoclonal antibody which identifies Band 3 and labeled, e.g., radiolabeled or enzyme labeled, monoclonal anti-idiotypic antibody to the aforesaid monoclonal antibody. Soluble *P. falciparum* in the test sample will interfere with the binding to the insolublized monoclonal antibody of the labeled monoclonal anti-idiotypic antibody and will thus decrease the amount of the detectable label, e.g., the radioactivity or the enzyme, bound by the insolublized antibody.

Non-limiting examples of supports for affinity-separation of antibodies, including monoclonals, include the following: activated Sepharose, activated cellulose and activated Sephadex. "Activated" refers to the creation, on the insoluble material, of reactive chemical groups that will form covalent linkages with the antibody molecules when incubated together under appropriate conditions. Typically, reactive groups are introduced into the insoluble substrate by the action of cyanogen bromide (CNBr) at high pH.

Significantly, as is well-known in the art, only a small portion of an antibody molecule, the paratope, is involved in the binding of the antibody to its epitope (see,

in general, Clark, W.R. (1986) The Experimental Foundations of Modern Immunology Wiley & Sons, Inc., New York; Roitt, I. (1991) Essential Immunology, 7th Ed., Blackwell Scientific Publications, Oxford). The pFc' and Fc regions, for example, are effectors of the complement cascade but are not involved in antigen binding. An antibody from which the pFc' region has been enzymatically cleaved, or which has been produced without the pFc' region, designated an $F(ab')_2$ fragment, retains both of the antigen binding sites of an intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an Fab fragment, retains one of the antigen binding sites of an intact antibody molecule. Fab fragments consist of a covalently bound antibody light chain and a portion of the antibody heavy chain denoted Fd. The Fd fragments are the major determinant of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity) and Fd fragments retain epitope-binding ability in isolation.

Within the antigen-binding portion of an antibody, there are complementary determining regions (CDRs), which directly interact with the epitope of the antigen, and framework regions (FRs), which maintain the tertiary structure of the paratope (see, in general, Clark, 1986; Roitt, 1991). In both the heavy chain Fd fragment and the light chain of IgG immunoglobulins, there are four framework regions (FR1 through FR4) separated respectively by three complementarity determining regions (CDR1 through CDR3). The CDRs, and in particular the CDR3 regions, and more particularly the heavy chain CDR3, are largely responsible for antibody specificity.

It is now well-established in the art that the non-CDR regions of a mammalian antibody may be replaced with similar regions of conspecific or heterospecific antibodies while retaining the epitopic specificity of the original antibody. This is most clearly manifested in the development and use of "humanized" antibodies in which non-human CDRs are covalently joined to human FR and/or Fc/pFc' regions to produce a functional antibody. See, e.g., U.S. patents 4,816,567, 5,225,539, 5,585,089, 5,693,762 and 5,859,205. Accordingly, humanized anti-Band 3 antibodies and the use of such antibodies (e.g., to provide passive immunity to a subject) are embraced with the inventions disclosed herein.

For example, PCT International Publication Number WO 92/04381 teaches the production and use of humanized murine RSV antibodies in which at least a portion of the murine FR regions have been replaced by FR regions of human origin. Such antibodies, including fragments of intact antibodies with antigen-binding ability, are often referred to as "chimeric" antibodies.

Thus, the present invention also provides for $F(ab')_2$, Fab, Fv and Fd fragments; chimeric antibodies in which the Fc and/or FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric $F(ab')_2$ fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; chimeric Fab fragment antibodies in which the FR and/or CDR1 and/or CDR2 and/or light chain CDR3 regions have been replaced by homologous human or non-human sequences; and chimeric Fd fragment antibodies in which the FR and/or CDR1 and/or CDR2 regions have been replaced by homologous human or non-human sequences. The present invention also includes so-called single chain antibodies and human monoclonal antibodies, such as those produced by mice having functional human immunoglobulin gene loci.

Such antibodies also may be used to identify tissues expressing protein or to purify protein. Antibodies, particularly the anti-idiotypic antibodies of the invention, also may be coupled to specific labeling agents for imaging or to anti-infectious agents, toxins such as ricin, other cytostatic or cytolytic drugs, and so forth, for therapeutic purposes.

As part of certain immunization compositions, one or more anti-Band 3 antibodies or stimulatory fragments thereof are administered with one or more adjuvants to induce an immune response or to increase an immune response. An adjuvant is a substance incorporated into or administered with antigen which potentiates the immune response. Adjuvants may enhance the immunological response by providing a reservoir of antigen (extracellularly or within macrophages), activating macrophages and stimulating specific sets of lymphocytes. Adjuvants of many kinds are well known in the art. Specific examples of adjuvants include monophosphoryl lipid A (MPL, SmithKline Beecham), a congener obtained after purification and acid hydrolysis of *Salmonella minnesota* Re 595 lipopolysaccharide;

saponins including QS21 (SmithKline Beecham), a pure QA-21 saponin purified from *Quillaja saponaria* extract; DQS21, described in PCT application WO96/33739 (SmithKline Beecham); QS-7, QS-17, QS-18, and QS-L1 (So et al., *Mol. Cells* 7:178-186, 1997); incomplete Freund's adjuvant; complete Freund's adjuvant; montanide; immunostimulatory oligonucleotides (see e.g. CpG oligonucleotides described by Kreig et al., *Nature* 374:546-9, 1995); and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol. Other adjuvants are known in the art and can be used in the invention (*see, e.g.* Goding, *Monoclonal Antibodies: Principles and Practice*, 2nd Ed., 1986). Methods for the preparation of mixtures or emulsions of peptide and adjuvant are well known to those of skill in the art of vaccination.

Other agents which stimulate the immune response of the subject can also be administered to the subject. For example, other cytokines are also useful in vaccination protocols as a result of their lymphocyte regulatory properties. Many other cytokines useful for such purposes will be known to one of ordinary skill in the art, including interleukin-12 (IL-12) which has been shown to enhance the protective effects of vaccines (*see, e.g., Science* 268: 1432-1434, 1995), GM-CSF and IL-18. Thus cytokines can be administered in conjunction with antigens and adjuvants to increase the immune response to the antigens.

There are a number of additional immune response potentiating compounds that can be used in vaccination protocols. These include costimulatory molecules provided in either protein or nucleic acid form. Such costimulatory molecules include the B7-1 and B7-2 (CD80 and CD86 respectively) molecules which are expressed on dendritic cells (DC) and interact with the CD28 molecule expressed on the T cell. This interaction provides costimulation (signal 2) to an antigen/MHC/TCR stimulated (signal 1) T cell, increasing T cell proliferation and effector function. B7 also interacts with CTLA4 (CD152) on T cells and studies involving CTLA4 and B7 ligands indicate that the B7-CTLA4 interaction can enhance antitumor immunity and CTL proliferation (Zheng et al., *Proc. Nat'l Acad. Sci. USA* 95:6284-6289, 1998).

The invention also contemplates gene therapy. The procedure for performing *ex vivo* gene therapy is outlined in U.S. Patent 5,399,346 and in exhibits submitted in the file history of that patent, all of which are publicly available documents. In

general, it involves introduction *in vitro* of a functional copy of a gene into a cell(s) of a subject which contains a defective copy of the gene, and returning the genetically engineered cell(s) to the subject. The functional copy of the gene is under operable control of regulatory elements which permit expression of the gene in the genetically engineered cell(s). Numerous transfection and transduction techniques as well as appropriate expression vectors are well known to those of ordinary skill in the art, some of which are described in PCT application WO95/00654. *In vivo* gene therapy using vectors such as adenovirus also is contemplated according to the invention.

Where it is desired to stimulate an immune response using a therapeutic composition of the invention, this may involve the stimulation of a humoral antibody response resulting in an increase in antibody titer in serum, a clonal expansion of cytotoxic lymphocytes, or some other desirable immunologic response. It is believed that doses of immunogens ranging from one nanogram/kilogram to 100 milligrams/kilogram, depending upon the mode of administration, would be effective. The preferred range is believed to be between 500 nanograms and 500 micrograms per kilogram. The absolute amount will depend upon a variety of factors, including the material selected for administration, whether the administration is in single or multiple doses, and individual patient parameters including age, physical condition, size, weight, and the stage of the disease. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

In addition to the above-described inventions which are based, in part, on the discovery of the particular Band 3 sequences which interact with MSP1 (or other malaria polypeptides of the invention), Applicants disclose herein related inventions which are based, in part, on the discovery of the particular portions of MSP1 (or other malaria polypeptides of the invention) that selectively binds to Band 3 protein. It is to be understood that enablement of the MSP1 (or other malaria polypeptides of the invention) related inventions throughout a broad scope can be accomplished in an analogous manner to that described for the Band 3 protein-related invention. Thus, the methods and definitions applied above in reference to the Band 3 molecule compositions and methods can be used in reference to the MSP1 molecule (or other malaria polypeptides of the invention) compositions and methods by substituting the

MSP1 molecule (or other malaria polypeptides of the invention) for the Band 3 molecule. For example, vectors expressing an MSP1 protein can be prepared by substituting an MSP1 nucleic acid for a Band 3 nucleic acid and inserting into an expression vector as described above. Thus, the invention embraces various compositions containing such MSP-1 peptides for use, e.g., in screening assays to detect the specific interaction between MSP-1 and Band 3 protein, as well as for use in diagnostic and therapeutic applications for detecting and treating, respectively, malaria infection. In general, such compositions contain components (or are contained in kits which contain additional components) which are selected to detect the specific interaction between MSP-1 (or other malaria polypeptides of the invention) and Band 3 protein (particularly, the Band 3 peptides disclosed herein).

According to one aspect of the invention, an isolated MSP1 peptide is provided. The peptide has an amino acid sequence selected from the group consisting of (Wellcome strain) SEQ ID NO:11 (MSP1(42)), SEQ ID NO:12 (MSP1(38), and SEQ ID NO:13 (MSP1(38)-N-terminal domain) and (FCB1 strain) $MSP1_{38}$ (SEQ ID NO:33), $MSP1_{42}$ (SEQ ID NO:34), and $MSP1_{19}$ (SEQ ID NO:35), and unique fragments thereof which bind Band 3 protein. The sequences for peptides SEQ ID NOs:11-13 are based on *P. falciparum* Wellcome strain: GenBank Accession no.X02919 as follows:

SEQ ID NO:11 (MSP1(42)) refers to X02919 amino acids 1262 to 1639, inclusive;

SEQ ID NO:12 (MSP1(38)) refers to X02919 amino acids 902 to 1261, inclusive; and

SEQ ID NO:13 (MSP1(38) – N terminal region) refers to X02919 amino acids 902 to 1121, inclusive.

The sequences for peptides SEQ ID Nos:33-35 are based on *P. falciparum* FCB1 strain. The sequence of polypeptide SEQ ID NO:33 is deposited as GenBank Accession No.:AF286876, amino acids 911-1263. The sequence for polypeptide SEQ ID NO:34 is deposited as GenBank Accession No: AF325919; amino acids 1264-1639. The sequence for polypeptide SEQ ID NO:35 is the C-terminal domain of MSP1(42); amino acids 1526-1639.

Such peptides may be contained in kits which detect the selective binding of the MSP1 peptide to a Band 3 protein, particularly to the Band 3 peptides disclosed herein.

According to another aspect of the invention, isolated nucleic acids encoding the foregoing MSP1 (or other malaria polypeptides of the invention) peptides are provided. The MSP1 nucleic acids have nucleotide sequences selected from the group consisting of (Wellcome strain) SEQ ID NO:54 (MSP1(42)), SEQ ID NO:55 (MSP1(38), and SEQ ID NO:56 (MSP1(38)-N-terminal domain) and (FCB1 strain) $MSP1_{38}$ (SEQ ID NO:57), $MSP1_{42}$ (SEQ ID NO:58), and $MSP1_{19}$ (SEQ ID NO:59), and unique fragments thereof. The sequences for nucleotide SEQ ID NOs:54-56 are based on *P. falciparum* Wellcome strain: GenBank Accession no.X02919. The sequences for nucleic acids SEQ ID Nos:57-59 are based on *P. falciparum* FCB1 strain, and are included in the nucleic acid sequences deposited as GenBank Accession Nos. AF286876 and AF325919

In certain embodiments, the nucleic acids comprise a unique fragment of the nucleotide sequence encoding the MSP1 peptides (or other malaria polypeptides of the invention) that selectively bind to Band 3. It is to be understood that all of the definitions described above in reference to the Band 3 molecules also apply to the MSP1 molecules (or other malaria polypeptides of the invention) of the invention. Thus, for example, the same definitions described above for "unique fragment", "isolated", "expression vectors", "alternate codons", and "operably joined" in reference to the Band 3 molecules of the invention apply to the MSP1 molecules (or other malaria polypeptides of the invention) of the invention. The expression vectors include the isolated foregoing MSP1 (or other malaria polypeptides of the invention) nucleic acids operably linked to a promoter. In another aspect of the invention, host cells transfected or transformed with the foregoing MSP1 (or other malaria polypeptides of the invention) nucleic acids or expression vectors are provided.

According to another aspect of the invention, compositions comprising the foregoing MSP1 (or other malaria polypeptides of the invention) peptides or nucleic acids and methods for making same are provided. The compositions are useful for inducing an immune response and include, e.g., immunogenic compositions. In general, the immunogenic compositions of the invention comprise one or more of the

foregoing isolated MSP1 (or other malaria polypeptides of the invention) polypeptides, a pharmaceutically acceptable carrier; and, optionally, an adjuvant, wherein the polypeptides are present in the composition in an effective amount to induce an immune system response. Antibodies to MSP1 peptides can be used, for example, in kits to identify agents which competitively bind to MSP1 and inhibit MSP1 binding to Band 3 protein or peptides as disclosed herein. According to a related aspect, a method of making a medicament (including, e.g., an immunogenic composition) is provided. The method involves placing one or more of the foregoing isolated MSP1 (or other malaria polypeptides of the invention) polypeptides or nucleic acids in a pharmaceutically acceptable carrier to form the medicament.

According to yet another aspect of the invention, a protein microarray comprising at least one isolated MSP-1 peptide selected from the group consisting of SEQ ID NOS. 11, 12, 13, 33, 34, and 35 is provided. Alternatively, a nucleic acid microarray comprising at least one nucleic acid encoding any of the foregoing MSP-1 peptides is provided. The arrays are useful in identifying agents which selectively bind to MSP1 molecules and inhibit MSP1 binding to Band 3 protein or peptides as disclosed herein.

According to still another embodiment, a method for identifying a candidate mimetic of an isolated MSP1 peptide is provided. The method involves: providing a Band 3 molecule (e.g., the above-described isolated Band 3 peptides) which binds an isolated MSP1 peptide (e.g., an MSP1 peptide having SEQ ID NOs:11, 12, 13, 33, 34, and/or 35); contacting the Band 3 molecule with the MSP1 peptide in the presence or absence of a test molecule; and determining the binding of the test molecule to the Band 3 molecule, wherein a test molecule which binds to the Band 3 molecule and inhibits binding of the Band 3 molecule with the MSP1 peptide is a candidate mimetic of the isolated peptide.

According to still another aspect of the invention, an antibody (or fragment thereof) (referred to herein as a "anti-MSP1 antibody") that: (1) selectively binds to any of the above-described peptides, particularly the peptides having a sequence selected from the group consisting of SEQ ID Nos. 11, 12, 13, 33, 34, and 35; and (2) inhibits binding of the MSP1 peptide to Band 3 protein (particularly the Band 3

peptides disclosed herein) is provided. Preferably, the antibody is a monoclonal antibody, and, more preferably, a humanized monoclonal antibody.

In addition to the inventions which are based, in part, on the discovery of the particular Band 3 sequences which interact with MSP1, Applicants disclose herein related inventions which are based, in part, on the discovery that Band 3 sequences interact with additional *Plasmodium* polypeptide sequences comprising the amino acid sequences selected from the group consisting of SEQ ID NOs:46-53. These polypeptides are encoded by the nucleic acid molecules comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:38-45. It is to be understood that enablement of the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA–related inventions throughout a broad scope can be accomplished in an analogous manner to that described for the Band 3 protein-related invention and the MSP1 polypeptide-related invention. Thus, the methods and definitions applied above in reference to the Band 3 and MSP-1 molecule compositions and methods can be used in reference to the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA molecule compositions and methods by substituting the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA molecule for the MSP-1 molecule. For example, vectors expressing an BBP-1 polypeptide can be prepared by substituting a BBP-1 nucleic acid for a MSP-1 nucleic acid and inserting into an expression vector as described above. Thus, the invention embraces various compositions containing such BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptides for use, e.g., in screening assays to detect the specific interaction between BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA and Band 3 protein, as well as for use in diagnostic (e.g. detection of the malaria polypeptides of the invention in biological samples obtained from a subject using conventional assays, such as an antibody-based assays or nucleic acid hybridization-based assays) and therapeutic applications (e.g., vaccines) for detecting and treating, respectively, malaria infection. The sequence identification for these polypeptides is provided in Table 1. In general, such compositions contain components (or are contained in kits which contain additional components) which are selected to detect the specific interaction between BBP-1, BBP-2, BBP-3, BBP-4,

BBP-5, BBP-6, RhopH3, and/or ABRA, and Band 3 protein (particularly, the Band 3 peptides disclosed herein).

Table 1. Band 3 Binding Polypeptide Identification

Polypeptide Name	Nucleic Acid SEQ ID NO.	Amino Acid SEQ ID NO.
BBP-1	38	46
BBP-2	39	47
BBP-3	40	48
BBP-4	41	49
BBP-5	42	50
BBP-6	43	51
RhopH3	44	52
ABRA	45	53

According to one aspect of the invention, an isolated BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA peptide is provided. The peptide has an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53 and unique fragments thereof that bind Band 3 protein. The sequences for these peptides are presented herein.

Such peptides may be contained in kits which detect the selective binding of the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide to a Band 3 protein, particularly to the Band 3 peptides disclosed herein.

According to another aspect of the invention, isolated nucleic acids encoding the foregoing BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptides are provided. In certain embodiments, the nucleic acids comprise a unique fragment of the nucleotide sequence encoding the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptides that selectively bind to Band 3 peptides. It is to be understood that all of the definitions described above in reference to the Band 3 and MSP-1 molecules also apply to the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA molecules of the invention. Thus, for example, the same definitions described above for "unique fragment", "isolated",

"expression vectors", "alternate codons", and "operably joined" in reference to the Band 3 and MSP-1 molecules of the invention apply to the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA molecules of the invention. The expression vectors include the isolated foregoing BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA nucleic acids operably linked to a promoter. In another aspect of the invention, host cells transfected or transformed with the foregoing BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA nucleic acids or expression vectors are provided.

According to another aspect of the invention, compositions comprising the foregoing BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptides or nucleic acids and methods for making same are provided. The compositions are useful for inducing an immune response and include, e.g., immunogenic compositions. In general, the immunogenic compositions of the invention comprise one or more of the foregoing isolated BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA polypeptides, a pharmaceutically acceptable carrier; and, optionally, an adjuvant, wherein the polypeptides are present in the composition in an effective amount to induce an immune system response. Antibodies to BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA polypeptides can be used, for example, in kits to identify agents which competitively bind to BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA and inhibit BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA binding to Band 3 protein or peptides as disclosed herein. According to a related aspect, a method of making a medicament (including, e.g., an immunogenic composition) is provided. The method involves placing one or more of the foregoing isolated BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptides or nucleic acids in a pharmaceutically acceptable carrier to form the medicament.

According to yet another aspect of the invention, a protein microarray comprising at least one isolated BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide selected from the group consisting of SEQ ID NOs:46-53 is provided. Alternatively, a nucleic acid microarray comprising at least one nucleic acid encoding any of the foregoing BBP-1, BBP-2, BBP-3, BBP-4, BBP-

5, BBP-6, RhopH3, and/or ABRA polypeptides is provided. The arrays are useful in identifying agents which selectively bind to BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA molecules and inhibit BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA binding to Band 3 protein or peptides as disclosed herein.

According to still another embodiment, a method for identifying a candidate mimetic of an isolated BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide is provided. The method involves: providing a Band 3 molecule (e.g., the above-described isolated Band 3 peptides) which binds an isolated BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide (e.g., a BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide having SEQ ID NOs:46-53); contacting the Band 3 molecule with the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide in the presence or absence of a test molecule; and determining the binding of the test molecule to the Band 3 molecule, wherein a test molecule which binds to the Band 3 molecule and inhibits binding of the Band 3 molecule with the isolated peptide is a candidate mimetic of the isolated peptide.

According to still another aspect of the invention, an antibody (or fragment thereof) (referred to herein as a "anti- BBP-1, anti-BBP-2, anti-BBP-3, anti-BBP-4, anti-BBP-5, anti-BBP-6, anti-RhopH3, and/or anti-ABRA antibody") that: (1) selectively binds to any of the above-described peptides, particularly the peptides having an amino acid sequence selected from the group consisting of SEQ ID Nos. 46-53; and (2) inhibits binding of the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide to Band 3 protein (particularly the Band 3 peptides disclosed herein) is provided. Preferably, the antibody is a monoclonal antibody, and, more preferably, a humanized monoclonal antibody.

The present invention also, in some aspects, involves the identification of cDNAs that encode *Plasmodium* polypeptides BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, and BBP-6. The sequence of the coding portion of the *Plasmodium* gene for each is presented as SEQ ID NOs:38-43, and the predicted amino acid sequences of these genes' protein products are presented as SEQ ID NOs:46-51.

The invention thus involves in one aspect *Plasmodium* BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, and BBP-6 polypeptides, nucleic acid molecules encoding those proteins, functional modifications and variants of the foregoing, useful fragments of the foregoing, as well as therapeutic and diagnostic products (including antibodies), non-human animal models, and methods relating thereto.

According to one aspect of the invention, isolated nucleic acid molecules are provided. The isolated nucleic acid molecule is selected from the group consisting of: An isolated nucleic acid molecule selected from the group consisting of:

(a) nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:38-43 and which codes for a *Plasmodium* polypeptide,

(b) deletions, additions and substitutions of the nucleic acid molecules of (a), which code for a *Plasmodium* polypeptide,

(c) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and

(d) complements of (a), (b) or (c).

The preferred isolated nucleic acids of the invention are *Plasmodium* nucleic acid molecules which encode a *Plasmodium* polypeptide. As used herein, a *Plasmodium* polypeptide refers to a protein that is encoded by a nucleic acid having SEQ ID NOs:38-45 and 54-59 or a functional fragment thereof, or a functional equivalent thereof (e.g., a nucleic acid sequence encoding the same protein as encoded by SEQ ID NOs:38-45 and 54-59), provided that the functional fragment or equivalent encodes a protein which exhibits a *Plasmodium* polypeptide functional activity. As used herein, a *Plasmodium* functional activity refers to the ability of a *Plasmodium* polypeptide of the invention to interact with a Band 3 molecule of the invention.

In the preferred embodiments, the isolated nucleic acid molecule is selected from the group consisting of SEQ ID NOs:38-45 and 54-59.

The invention provides isolated nucleic acid molecules which code for *Plasmodium* proteins and which hybridize under stringent conditions to a nucleic acid molecule consisting of the nucleotide selected from the group consisting of SEQ ID

NOs:38-45 and 54-59. Such nucleic acids may be DNA, RNA, composed of mixed deoxyribonucleotides and ribonucleotides, or may incorporate synthetic non-natural nucleotides. Various methods for determining the expression of a nucleic acid and/or a polypeptide in cells are known to those of skill in the art and are described further below. As used herein, the term protein is meant to include large molecular weight proteins and peptides and low molecular weight peptides or fragments thereof.

The term "stringent conditions" as used herein refers to parameters with which the art is familiar. Nucleic acid hybridization parameters may be found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. More specifically, stringent conditions, as used herein, refers, for example, to hybridization at 65°C in hybridization buffer (3.5 x SSC, 0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% Bovine Serum Albumin (BSA), 2.5mM NaH_2PO_4 (pH 7), 0.5% SDS, 2mM EDTA). SSC is 0.15M sodium chloride/0.15M sodium citrate, pH 7; SDS is sodium dodecyl sulphate; and EDTA is ethylenediaminetetracetic acid. After hybridization, the membrane upon which the DNA is transferred is washed at 2x SSC at room temperature, and then at 0.1x SSC/0.1 x SDS at temperatures up to 68°C.

The foregoing set of hybridization conditions is but one example of stringent hybridization conditions known to one of ordinary skill in the art. There are other conditions, reagents, and so forth which can be used, which result in a stringent hybridization. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles of *Plasmodium* nucleic acid molecules and Band 3 nucleic acid molecules of the invention. The skilled artisan also is familiar with the methodology for screening cells and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing.

In general homologs and alleles of the malaria polypeptides typically will share at least 40% nucleotide identity and/or at least 50% amino acid identity to SEQ

ID NOs:38-45 and 54-59 and SEQ ID NOs:46-53, 11-13, and 33-35, respectively, in some instances will share at least 50% nucleotide identity and/or at least 65% amino acid identity and in still other instances will share at least 60% nucleotide identity and/or at least 75% amino acid identity. Preferred homologs and alleles share nucleotide and amino acid identities with SEQ ID NOs:38-45, 54-59 and SEQ ID NOs:46-53, 11-13, and 33-35, respectively, and encode polypeptides of greater than 80%, more preferably greater than 90%, still more preferably greater than 95% and most preferably greater than 99% identity. The percent identity can be calculated using various publicly available software tools developed by NCBI (Bethesda, Maryland) that can be obtained through the internet (ftp:/ncbi.nlm.nih.gov/pub/). Exemplary tools include the BLAST system available at http://www.ncbi.nlm.nih.gov, which uses algorithms developed by Altschul et al. (*Nucleic Acids Res.* 25:3389-3402, 1997). Pairwise and ClustalW alignments (BLOSUM30 matrix setting) as well as Kyte-Doolittle hydropathic analysis can be obtained using the MacVector sequence analysis software (Oxford Molecular Group). Watson-Crick complements of the foregoing nucleic acid molecules also are embraced by the invention.

In screening for *Plasmodium* genes, a Southern blot may be performed using the foregoing conditions, together with a detectably labeled probe (e.g. radioactive or chemiluminescent probes). After washing the membrane to which the DNA is finally transferred, the membrane can be placed against X-ray film or a phosphorimager to detect the radioactive or chemiluminescent signal. In screening for the expression of *Plasmodium* RNA, Northern blot hybridizations using the foregoing conditions can be performed on samples taken from cells or subjects suspected of having a *Plasmidium* infection. Amplification protocols such as PCR using primers that hybridize to the sequences presented also can be used for detection of the *Plasmodium* genes or expression thereof.

Identification of related sequences can be achieved using PCR and other amplification techniques suitable for cloning related nucleic acid sequences. Preferably, PCR primers are selected to amplify portions of a nucleic acid sequence believed to be conserved (e.g., a binding domain, etc.). Again, nucleic acids are preferably amplified from a *Plasmodium* library.

The invention also includes degenerate nucleic acid molecules which include alternative codons to those present in the native materials. For example, serine residues are encoded by the codons TCA, AGT, TCC, TCG, TCT and AGC. Each of the six codons is equivalent for the purposes of encoding a serine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the serine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, *in vitro* or *in vivo*, to incorporate a serine residue into an elongating *Plasmodium* polypeptide. Similarly, nucleotide sequence triplets that encode other amino acid residues include, but are not limited to: CCA, CCC, CCG and CCT (proline codons); CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); ACA, ACC, ACG and ACT (threonine codons); AAC and AAT (asparagine codons); and ATA, ATC and ATT (isoleucine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

According to another aspect of the invention, further isolated nucleic acid molecules that are based on the above-noted *Plasmodium* nucleic acid molecules are provided. In this aspect, the isolated nucleic acid molecules are selected from the group consisting of:

(a) a unique fragment of the nucleotide sequence selected from the group consisting of:

nucleotides 1-1287 of SEQ ID NO:38 between 12 and 1286 nucleotides in length, nucleotides 1-3576 of SEQ ID NO:39 between 12 and 3557 nucleotides in length, nucleotides 1-903 of SEQ ID NO:40 between 12 and 902 nucleotides in length, nucleotides 1-1203 of SEQ ID NO:41 between 12 and 1202 nucleotides in length, nucleotides 1-3996 of SEQ ID NO:42 between 12 and 3995 nucleotides in length, and nucleotides 1-876 of SEQ ID NO:43 between 12 and 875 nucleotides in length, and nucleotides 1-2712 of SEQ ID NO:44 between 12 and 2711 nucleotides in length, and nucleotides 1-2232 of SEQ ID NO:45 between 12 and 2231 nucleotides in length, and nucleotides 1-1134 of SEQ ID NO:54 between 12 and 1133 nucleotides in length, and nucleotides 1-1080 of SEQ ID NO:55 between 12 and 1079 nucleotides in length, and nucleotides 1-660 of SEQ ID NO:56 between 12 and 659 nucleotides in length,

and nucleotides 1-1080 of SEQ ID NO:57 between 12 and 1079 nucleotides in length, and nucleotides 1-1131 of SEQ ID NO:58 between 12 and 1130 nucleotides in length, and nucleotides 1-343 of SEQ ID NO:59 between 12 and 342 nucleotides in length, and

(b) complements of (a),

wherein the unique fragments exclude nucleic acids having nucleotide sequences that are contained within SEQ ID NOs:38-45, 54-59, and that are known as of the filing date of this application.

The invention also provides isolated unique fragments of SEQ ID NOs:38-44 or complements of SEQ ID NOs:38-45, 54-59. A unique fragment is one that is a 'signature' for the larger nucleic acid. It, for example, is long enough to assure that its precise sequence is not found in molecules outside of the *Plasmodium* nucleic acid molecules defined above. Those of ordinary skill in the art may apply no more than routine procedures to determine if a fragment is unique within the *Plasmodium*. Unique fragments, however, exclude fragments completely composed of the nucleotide sequences that are contained within SEQ ID NO:38-45, 54-59 and that are known as of the filing date of this application.

Unique fragments can be used as probes in Southern blot, Northern blot, and Gene Chip/microarray assays to identify such nucleic acid molecules, or can be used in amplification assays such as those employing PCR. As known to those skilled in the art, large probes such as 200 nucleotides or more are preferred for certain uses such as Southern blots, while smaller fragments will be preferred for uses such as in PCR and Gene Chip/microarray assays. Unique fragments also can be used to produce fusion proteins for generating antibodies or determining binding of the polypeptide fragments, or for generating immunoassay components. Likewise, unique fragments can be employed to produce nonfused fragments of the *Plasmodium* polypeptides that are useful, for example, in the preparation of antibodies in immunoassays. Unique fragments further can be used as antisense molecules to inhibit the expression of *Plasmodium* nucleic acids and polypeptides, particularly for therapeutic purposes as described in greater detail below.

As will be recognized by those skilled in the art, the size of the unique fragment will depend upon its conservancy in the genetic code. Thus, some regions

of SEQ ID NOs:38-44, 54-59 and their complements will require longer segments to be unique while others will require only short segments, typically between 12 and 32 nucleotides or more in length (e.g. 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, and 32 or more), up to the entire length of the disclosed sequence. Many segments of the polynucleotide coding region or complements thereof that are 18 or more nucleotides in length will be unique. Those skilled in the art are well versed in methods for selecting such sequences, typically on the basis of the ability of the unique fragment to selectively distinguish the sequence of interest from non-*Plasmodium* nucleic acid molecules. A comparison of the sequence of the fragment to those on known data bases typically is all that is necessary, although *in vitro* confirmatory hybridization and sequencing analysis may be performed.

A unique fragment can be a functional fragment. A functional fragment of a nucleic acid molecule of the invention is a fragment which retains some functional property of the larger nucleic acid molecule, such as coding for a functional polypeptide, binding to proteins, regulating transcription of operably linked nucleic acid molecules, and the like. One of ordinary skill in the art can readily determine using the assays described herein and those well known in the art to determine whether a fragment is a functional fragment of a nucleic acid molecule using no more than routine experimentation.

As used herein, the term "antisense oligonucleotide" or "antisense" describes an oligonucleotide that is an oligoribonucleotide, oligodeoxyribonucleotide, modified oligoribonucleotide, or modified oligodeoxyribonucleotide which hybridizes under physiological conditions to DNA comprising a particular gene or to a transcript of that gene and, thereby, inhibits the transcription of that gene and/or the translation of that mRNA. The antisense molecules are designed so as to interfere with transcription or translation of a target gene upon hybridization with the target gene or transcript. Those skilled in the art will recognize that the exact length of the antisense oligonucleotide and its degree of complementarity with its target will depend upon the specific target selected, including the sequence of the target and the particular bases which comprise that sequence. It is preferred that the antisense oligonucleotide be constructed and arranged so as to bind selectively with the target under physiological conditions, i.e., to hybridize substantially more to the target sequence than to any

other sequence in the target cell under physiological conditions. Based upon SEQ ID NOs:38-45, 54-59, or upon allelic or homologous genomic and/or cDNA sequences, one of skill in the art can easily choose and synthesize any of a number of appropriate antisense molecules for use in accordance with the present invention. In order to be sufficiently selective and potent for inhibition, such antisense oligonucleotides should comprise at least 10 and, more preferably, at least 15 consecutive bases which are complementary to the target, although in certain cases modified oligonucleotides as short as 7 bases in length have been used successfully as antisense oligonucleotides (Wagner et al., *Nature Biotechnology* 14: 840-844, 1996).

Most preferably, the antisense oligonucleotides comprise a complementary sequence of 20-30 bases. Although oligonucleotides may be chosen which are antisense to any region of the gene or its transcripts, in preferred embodiments the antisense oligonucleotides correspond to N-terminal or 5' upstream sites such as translation initiation, transcription initiation, or promoter sites. In addition, 3'-untranslated regions may be targeted. Targeting to mRNA splicing sites also has been used in the art but may be less preferred because alternative mRNA splicing of the *Plasmodium* transcript occurs. In addition, the antisense is targeted, preferably, to sites in which mRNA secondary structure is not expected (see, e.g., Sainio et al., *Cell Mol. Neurobiol.* 14(5):439-457, 1994) and at which proteins are not expected to bind. The present invention also provides for antisense oligonucleotides which are complementary to genomic DNA and/or cDNA corresponding to SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, and/or SEQ ID NO:59. Antisense to allelic or homologous cDNAs and genomic DNAs are enabled without undue experimentation.

In summary, the various aspects of the invention include one or more of the following utilities:

1. Treatment and prevention of malaria disease in human and animals:

(a) Development of a peptide, peptidomimetic, and/or protein antimalarial drugs partially or entirely based on the human Band 3 protein residues 720-761 and/or 807-826.

(b) Development of an antimalarial gene therapy using DNA plasmids encoding Band 3 protein sequence derived partially or entirely from the human Band 3 protein residues 720-761 and/or 807-826.

(c) Development of a peptide, peptidomimetic, and/or protein antimalarial drug using three dimensional structure information of human Band 3 protein containing a partial or the entire amino acid sequence of residues 720-761 and/or 807-826.

(d) Development of a non-peptide, non-protein, and/or non-peptidomimetic antimalarial drug derived from three dimensional structure information of human Band 3 protein containing a partial or the entire amino acid sequence of residues 720-761 and 807-826.

(e) Development of an idiotype protein vaccine that produces anti-idiotypic antibodies mimicking the entire or partial structure of residues 720-761 and/or 807-826 of the human Band 3 protein, for use in malaria disease.

(f) Development of an idiotype DNA vaccine in which DNA encodes the idiotypic determinants to induce the production of such anti-idiotypic antibodies as defined above.

(g) Use of a non-human erythrocyte Band 3 gene and/or protein sequence corresponding to the human Band 3 protein residues 720-761 and/or 807-826 for the purpose of developing drug or vaccine for human and/or animal malaria disease.

(h) Use of a gene and/or protein sequence corresponding to the MSP-1, BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, ABRA molecules of the invention for the purpose of developing drugs or vaccines for human and/or animal malaria disease and for malaria diagnostic purposes.

2. Screening of the malaria parasite ligand(s) binding to the host Band 3 receptor:

(a) Use of a peptide and/or protein containing partial or entire sequence of human Band 3 protein residues 720-761 and/or 807-826 in efforts to identify and/or develop drug or vaccine for human and/or animal malaria disease. These include experiments including, but not limited to: protein or peptide binding experiments carried out *in vitro* and *in vivo*; three-dimensional structure-based approaches; computer modeling; combinatorial chemistry screening; other high throughput screening approaches. The malaria parasite ligand(s) identified and/or characterized by utilizing the inventions

disclosed herein as new targets for the development of a highly efficient malaria vaccine and/or drug.

(b) Use of nucleotide sequence encoding partial or entire amino acid sequence of human Band 3 protein residues 720-761 and/or 807-826 to identify and/or functionally characterize the malaria parasite ligand(s) binding to the erythrocyte Band 3 protein.

(c) Use of a non-human erythrocyte Band 3 gene and/or protein sequence corresponding to the human Band 3 protein residues 720-761 and/or 807-826 for the purpose of carrying out the screening of malaria parasite ligand as described in (a) and (b) above.

3. Screening Assays to select agents which inhibit MSP1 binding to Band 3 protein, and screening assays to select agents that inhibit BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA binding to Band 3 protein.

(a) Use of a peptide and/or protein containing partial or entire sequence of MSP1, BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and/or ABRA polypeptide in combination with a peptide and/or protein containing human Band 3 protein (e.g., Band 3 residues 720-761 and/or 807-826) to identify and/or develop drug or vaccine for human and/or animal malaria disease. These include experiments including, but not limited to: protein or peptide binding experiments carried out *in vitro* and/or *in vivo*; three-dimensional structure-based approaches; computer modeling; combinatorial chemistry screening; other high throughput screening approaches. These screening assays can be used to detect lead molecules in mixtures (e.g., libraries) of synthetic or naturally-occurring molecules.

Examples

Introduction to the Examples:

To extend our observation that the erythrocyte Band 3 (-/-) mice are completely resistant to invasion by murine malaria parasite *Plasmodium yoelii* 17 XL, we have conducted a series of experiments to identify the key host receptor that mediates malaria parasite invasion into human red blood cells. Using a peptide scanning technique, we have identified two specific regions of the erythroid Band 3 protein (also known as Anion Exchanger 1 or AE1) that serve as the receptor for

malaria parasite invasion into the human erythrocytes. These two regions are located within ectoplasmic domains 5 and 6 of the Band 3 protein. In our study, the ectoplasmic domains 5 and 6 are defined as below based on the published human Band 3 amino acid sequence

Ectoplasmic domain 5 (amino acid residues 720-761): (SEQ ID NO:22)
GMPWLSATTVRSVTHANALTVMGKASTPGAAAQIQEVKEQRI

Ectoplasmic domain 6 (amino acid residues 807-857): (SEQ ID NO:23)
DRILLLFKPPKYHPDVPYVKRVKTWRMHLFTGIQIICLAVLWVVKSTPASL

Four peptides ($B3_{5A}$, $B3_{5B}$, $B3_{5C}$, $B3_{6A}$) derived from ectoplasmic domains 5 and 6 of the Band 3 protein inhibited the invasion of the most lethal *Plasmodium falciparum* malaria parasite into human erythrocytes *in vitro*, while other peptides used in the scanning experiment had no significant effect as compared to the control sample. The peptides $B3_{5C}$ and $B3_{6A}$ showed highest inhibition amongst all peptides tested. The amino acid sequences of the four peptides are:

$B3_{5A}$ (720-739): GMPWLSATTVRSVTHANALT (SEQ ID NO:1)
$B3_{5B}$ (731-750): SVTHANALTVMGKASTPGAA (SEQ ID NO:2)
$B3_{5C}$ (742-761): GKASTPGAAAQIQEVKEQRI (SEQ ID NO:3)
$B3_{6A}$ (807-826): DRILLLFKPPKYHPDVPYVK (SEQ ID NO:4)

The blot overlay technique using the peptides $B3_{5C}$ and $B3_{6A}$ revealed that these peptides specifically bind to a set of human *P. falciparum* malaria proteins expressed at the merozoite stage. One of the proteins identified by the blot overlay assay corresponds to the molecular mass of Merozoite Surface Protein-1 (MSP-1), a well known malaria parasite protein.

We have further studied the interaction between the Band 3 peptides and MSP1 and have identified sequences within MSP1 which selectively bind to the Band 3 receptor. These studies and sequences are described in detail below. We have also examined the interaction between the Band 3 polypeptides and BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA polypeptides. These studies are described in more detail below. The amino acid sequences of the BBP-1, BBP-2, BBP-3, BBP-4, BBP-5, BBP-6, RhopH3, and ABRA polypeptides are SEQ ID NOs:46-53. These polypeptides of the invention are encoded by the nucleic acid molecules comprising nucleotide sequences selected from the group consisting of SEQ ID NOs:38-45.

Based on our findings, we propose an parasite invasion pathway model illustrated in Figure 6.

EXAMPLE 1. Anti-Band 3 Antibody Preparation

Using the method of G. Kohler and C. Milstein, Nature 256, 495-496, (1975), a BALB/c mouse is immunized with washed human red cells expressing Band 3 or isolated Band 3 by weekly intraperitoneal administration of approximately 10^7 erythrocytes each or an equivalent amount of the isolated Band 3 protein. The spleen of the mouse is then removed and a cell suspension prepared in tissue culture medium (RPMI-1640 with additional glutamine, 5mM). The spleen cell suspension is mixed with a suspension of the mouse myeloma cell line P3/NSO-Ag4-1 (NS-0) (obtained from the ATCC) which, being deficient in the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPRT), will not grow in tissue culture media containing hypoxanthine, aminopterin and thymidine (HAT media).

The mixture contains four spleen cells to one myeloma cell. Fusion is promoted by the addition of polyethylene glycol (PEG) of an average molecular weight of 2000. After fusion, the cells are washed free of PEG, resuspended in HAT medium and allowed to grow to a density of 10^6 live cells per ml and aliquots of 0.1 ml added to the wells of a 24-well tray containing feeder cells (from BALB/c thymus).

Partial changes of culture medium are performed at approximately 3, 5 and 7 days and the supernatants are removed approximately 14 days postfusion and tested for the presence of antibodies that bind to Band 3. Since the process is conducted in the presence of HAT, essentially non-fused myeloma cells are dead at this time, which prevents them from possibly overgrowing the fused (hybrid) ones.

The unfused spleen cells are also dead because of their very limited capacity to grow ex vivo in this tissue culture medium. The hybrid cells grow and multiply because the normal spleen cells contribute the enzyme HGPRT and the myelomatous cells for the capacity for indefinite proliferation. The supernatants from the wells containing colonies of hybrid cells are assayed on a panel including isolated Band 3 (e.g., immobilized in microtiter plate wells). The cellular contents of the positive

well(s) that contain antibodies to Band 3 are then recovered and suspended to a concentration of 3 cells per ml.

Aliquots of 0.1 ml are then added to fresh wells, so that on the average only one of every three wells receives a cell and, thus, the colonies that result from the growth of this very diluted suspension are likely to be true "clones", i.e., descendents from a single progenitor. When the colonies attained a size of 10^2 cells their supernatants are again screened for the presence of Band 3 antibodies and the most strongly positive ones are allowed to expand to a number of 10^5 to 10^6. Dilution of these cell suspensions to a concentration of 3 cells/ml and plating volumes of 0.1 ml as before results in the growth of doubly-cloned hybrid, antibody-producing cell lines.

The concentration of monoclonal antibody at the time of maturity of a culture flask is 20 +/-.5 μg/ml. The cloned hybridoma also grows *in vivo* in mice of the BALB/c strain or of its first generation (Fl) hybrids. This growth is in the form of malignant myelomatous tumors. When live hybridoma cells are injected into susceptible animals, they secrete high concentrations of antibody into the peritoneal spaces. "Priming" the animals with irritants such as incomplete Freund's adjuvant or Pristane injected into the peritoneum, before grafting the hybridomas, results in the formation of large volumes of ascites containing antibody in concentrations higher than 5 mg/ml.

EXAMPLE 2. Anti-Band 3 Antibody Blocks Penetration of *P. falciparum* Merozoites into Erythrocytes.

The methods disclosed herein are based on those disclosed in U.S. patent no. 5,101,017, which reportedly are adapted from those described in L. H. Miller, S. J. Mason, J. A. Dvorak, T. Shiroishi and M. H. McGinnis, "Erythrocyte Receptors for Malarial Merozoites and the Duffy Blood Group System", Human Blood Groups, 5th International Convocation on Immunology, Buffalo, NY, 1976, Basel Karger, pp. 394-400, 1977.

Standard numbers of merozoites are incubated with standard numbers of erythrocytes. In parallel wells, red cells of primates of different species are exposed to *P. falciparum* in the presence of an Anti-Band 3 antibody or non-specific antibody as a control, e.g., anti-Rh29 and anti-Kl4 antibodies (where are reactive with

essentially all human and most primate red blood cells, but their antigenic epitopes are unrelated to Band 3). A third well for each erythrocyte donor contains only tissue culture medium instead of monoclonal antibodies. The two latter, control wells allow the determination of the proportion of red cells that are "normally" penetrated by the parasite under these conditions. Thus, comparison between this proportion and that in the well containing the anti-Band 3 monoclonal antibody permits estimation of its inhibitory effect

EXAMPLE 3. Specificity Determination.

The anti-Band 3 monoclonal antibody is tested for its capacity to immunoprecipitate or otherwise selectively bind to the Band 3 protein and/or Band 3 peptides of the invention (e.g., immobilized, labeled with a detectable agent). For example, red blood cell membrane proteins (including Band 3) or isolated Band 3 protein or peptides of the invention, separated by SDS-PAGE and blotted onto nitrocellulose filters, are exposed to labeled monoclonal antibody molecules and a single Band of the appropriate molecular size (of the Band 3 protein or peptides of the invention) and overall chemical characteristics is obtained.

EXAMPLE 4. Preparation of Monoclonal Anti-idiotypic Antibodies.

All antibody molecules are, at the same time, antigens since their ability to function as antibodies, i.e., to bind to antigen, depends on a special stereochemical configuration which is specific for each antibody and is called an "idiotype". A monoclonal antibody immunoglobulin is constituted of exactly identical molecules, each having the same specific combining site, which, being complementary to the respective antigen, becomes antigenic for the antibody-producing host and to other animals of the same strain. In other words, the idiotype of an antibody leads to the production of anti-idiotypic antibodies. This antigenic property can thus be used to elicit such anti-idiotypic antibodies by injecting naive hosts with purified monoclonal antibodies produced in animals of the same inbred strain.

This procedure is based on that described in U.S. patent no. 5,101,017. The preparation of anti-idiotypic antibodies is accomplished by first purifying the original monoclonal antibody (designated Abl) by affinity chromatography, emulsifying it in

complete Freund's adjuvant and injecting this emulsion into the peritoneum and under the skin in multiple sites of BALb/c mice. A second, identical injection is given approximately two weeks later. Subsequent injections require the use of incomplete Freund's adjuvant. The schedule of these injections and the quantities of immunoglobulin injected are empirical and different procedures have been reported to be successful (e.g., two further injections in complete Freund's can be given two weeks apart and following the initial two injections in complete Freund's adjuvant by two weeks). The recipient mice are rested for two months followed by two bi-weekly injections of the Anti-Band 3 antibody (Abl). One week later, the spleens are removed and fused with NSO myeloma cells. Hybridomas are grown as described elsewhere in this application and screening is performed by competitive inhibition of Abl binding to human red cells which express Band 3 or screening by competitive inhibition of Ab1 binding to isolated Band 3 or a Band 3 peptide of the invention. This inhibition test consists of adding the supernatants of hybridomas putatively producing anti-idiotypic antibodies (Ab2) to a dilution of Anti-Band 3 antibody (Ab1) and allowing the mixture to react with Band 3 (isolated or expressed by an erythrocyte or other cell). The presence of Ab2 inhibits that reaction. Confirmation of the specificity of presumptive anti-idiotypic antibody produced by the hybridomas is conducted by measuring its binding to red blood cells (there should be none) and the inhibition of monoclonal antibodies of the unrelated specificities: Rh29, K2, Kl4, M, N, B and Wr.sup.b (again there should be none). All these control tests being negative, the cells making the anti-anti-Band 3 antibody are cloned by limiting dilution.

These Ab2-producing clones (anti-anti-Band 3 ab) are then expanded and used to produce large amounts of supernatant and ascitic fluids. Ab2 binding to, and inhibition of the red cell penetration by, *P. falciparum* merozoites in subsequent experiments demonstrates that the epitope recognized by the anti-anti-Band 3 monoclonal antibody is indeed the site used by *P. falciparum* since the parasite shares the binding structure of the monoclonal antibody.

Thus, using standard immunological, combinatorial chemistry, and three dimensional structural approaches, novel compounds are identified that specifically inhibit the invasion of the malaria parasite into host erythrocytes. The development of

these compounds is based on the structure of ectoplasmic domains 5 and 6 of the Band 3 protein, the receptor region as defined by the amino acid sequence of the peptides $B3_{5A}$, $B3_{5B}$, $B3_{5C}$, and $B3_{6A}$, and their corresponding MSP-1 ligand(s) on the merozoite surface.

EXAMPLE 5 Band 3 Is The Host Receptor Binding Merozoite Surface Protein-1 in the Malaria Parasite *Plasmodium Falciparum* Invasion of Red Blood Cells

Methods

Synthetic Band 3 Peptides

Human Band 3 peptides were synthesized with an N-terminal biotin tag and purified to homogeneity by HPLC (Peptide 1, amino acid 424-435; 2, 477-491; 3A, 538-557; 3B, 551-570; 4A, 623-642; 4B, 634-653; 4C, 644-663; 5A, 720-739; 5B, 731-750; 5C, 742-761; 6A, 807-826; 6B, 823-842; 6C, 838-857). Peptides were initially solubilized with minimal DMSO and serially diluted with either PBS or pertinent buffer to give $\leq$ 1% DMSO final concentration in all subsequent assays. Peptide 6C was not soluble under these conditions and could not be used in the study.

Parasite Culture and Infection Determination

Plasmodium parasite cultures were maintained at 37°C as described by Klotz, F.W., et. al., *J Exp Med* 165:1713-1718, 1987. To determine infection in RBCs, rings were counted from RBCs in Geimsa-stained thin smears.

Recombinant $MSP1_{38}$, $MSP1_{42}$ and $MSP1_{19}$

$MSP1_{38}$ (SEQ ID NO:33), $MSP1_{42}$ (SEQ ID NO:34), and $MSP1_{19}$ (SEQ ID NO:35) genes were amplified from a *P. falciparum* (FCB1 strain) cDNA library (J. B. Dame) by PCR using the following primers: 5'-CTCGAGCTCAGGATAAACCC (SEQ ID NO:14, $MSP1_{38}$, sense, 3121-3133, *Xho*I), 5'-GCGGCCGCACTTGTTAGT (SEQ ID NO:15, $MSP1_{38}$, antisense, 4200-4193, *Not*I), 5'-CTCGAGCTGGAGAAGCAGTAACT (SEQ ID NO:16, $MSP1_{42}$, sense, 4201-4218, *Xho*I), 5'-GCGGCCGCACTAAATGAAACTGTATA (SEQ ID NO:17, $MSP1_{42}$, antisense, 5334-5321 *Not*I), 5'-CCGGGATCCAACATTTCACAACACCAA: (SEQ ID NO:18, $MSP1_{19}$, sense, 4993-5009 *Bam*HI), 5'-

CCGGAATTCAATGAAACTGTATAATA (SEQ ID NO:19, $MSP1_{19}$, antisense, 5334-5318, *Eco*RI). Similar cDNA libraries as well as cDNA pools are available to the public from MR4, ATCC (www.malaria.atcc.org).

Primer sequences were based on the Wellcome strain of *P. falciparum* MSP1 (Holder, A.A., et al., *Nature* 317:270-273, 1985; Miller, L.H., et al., *Mol Biochem Parasitol* 59:1-14, 1993). $MSP1_{38}$ and $MSP1_{42}$ were cloned into pGEX6P-2 (Amersham Pharmacia Biotech, Piscataway, NJ), and $MSP1_{19}$ was cloned into pGEX-2TK (Amersham Pharmacia Biotech) using restriction sites indicated above. All three MSP1 domains were expressed as GST-fusion proteins in *E. coli* DH5α, affinity purified using GSH beads. ^{32}P-labeled $MSP1_{19}$ was obtained by radiolabeling GST-$MSP1_{19}$ with ^{32}P-γ-ATP and removing the GST domain with thrombin (as described in Manufacturer's instructions).

Recombinant Band 3 Peptides 5ABC, 5BC, and 6AB

The 5ABC (amino acid 720-761), 5BC (731-761), and 6AB (807-842) genes were amplified from a human reticulocyte cDNA library by PCR and cloned into pGEX-2TK (Pharmacia) as GST fusion proteins. Primers: 5'-CCGGGATCCGGGATGCCCTGGCTCAGTGCCA (SEQ ID NO:20, 5ABC, sense, 2272-2293, *Bam*HI), 5'-CCGGAATTCTTAGATCCGCTGCTCTTTGACCTC (SEQ ID NO:21, 5ABC and 5BC, antisense, 2397-2377, *Eco*RI), 5'-CCGGGATCCTCCGTCACCCATGCCAACGCC (SEQ ID NO:24, 5BC, sense, 2305-2325, *Bam*HI), 5'-CCGGGATCCGACCGCATCTTGCTTCTGTTCA (SEQ ID NO:25, 6AB, sense, 2533-2554, *Bam*HI), and 5'-CCGGAATTCTTAGATCTGGATGCCCGTGAA (SEQ ID NO:26, 6AB, antisense, 2640-2620, *Eco*RI). GST-5ABC and GST-5BC were expressed, affinity purified, and radiolabeled with ^{32}P as above to obtain ^{32}P-labeled 5ABC and 5BC. GST-6AB expressed under same conditions was not soluble and could not be used in the study.

Blot Overlay Assay

Naturally released *P. falciparum* (3D7 strain) merozoites isolated as described (Mrema, J.E., et al., *Exp Parasitol* 54:285-295, 1982) and human RBC ghosts prepared as described (Dodge, J.T., et al., *Arch Biochem Biophys* 100:119-130, 1963)

were subjected to SDS-PAGE. Proteins transferred onto nitrocellulose membrane were blocked overnight with 10% milk, 2% BSA, TBST (0.05%) at 4°C. After washing (4X) in TBST, the blot was incubated with biotinylated Band 3 peptides (each 400 μM) in TBST (0.025%), 10 mM phosphate buffer (pH 8.0), 60 mM KCl for 4 h at RT. After extensive washing, the blot was incubated with neutravidin-linked horseradish peroxidase (1:21,000, Pierce Chemical Co., Rockford, IL) in TBST (0.05%) with 2% BSA for 5-6 h at RT. After washing the blot with TBST (5X) and TBS (2X), bound peptides were visualized by the ECL method (Pierce Chemical Co.).

Native MSP1 Binding to mAb 5.2 and 5ABC

Radiolabeled parasite protein extract (180 μl) was incubated with either mAb 5.2 (20 μl) or GST-5ABC beads (40 μl, 50% slurry) for 22-24 h at 4°C. Protein G agarose beads (50 μl, 50% slurry) were added to the former mixture and further incubated for 3h. In both samples, beads were washed with PBS (2X) and proteins associated with beads were analyzed by SDS-PAGE followed by Coomassie staining and autoradiography. GST beads were used as control.

Yeast Two-Hybrid Assay

The 5ABC, 5BC, and 6AB genes were amplified by PCR as above and cloned into pGBKT7 (CLONTECH Laboratories, Inc., Palo Alto, CA). Primers were the same as the above except *Eco*RI (sense) and *Bam*HI (antisense) restriction sites were used. $MSP1_{38a}$, $MSP1_{38b}$, $MSP1_{42}$, and $MSP1_{19}$ constructs were prepared by amplifying the MSP1 gene by PCR using the same template as above and cloned into pGADT7 (Clontech). Primers used were: 5'-GGCCATATGGATGATACATCACATT (SEQ ID NO:27, $MSP1_{38a}$, sense, 3148-3163, *Nde*I), 5'-GGCCTCGAGGTTTTCTAAACTGGCAT (SEQ ID NO:28, $MSP1_{38a}$, antisense, 3780-3764, *Xho*I), 5'-GGCCATATGTTTAAAGTATTAAGTA (SEQ ID NO:29, $MSP1_{38b}$, sense, 3781-3796, *Nde*I), 5'-GGCCTCGAGTTCTCCTGTTACTACTTG (SEQ ID NO:30, $MSP1_{38b}$, antisense, 4206-4189, *Xho*I), 5'-GCCGAATTCGCAGTAACTCCTTCCG (SEQ ID NO:31, $MSP1_{42}$, sense, 4207-4222, *EcoR*I) 5'-GCCGGATCCAATGAAACTGTATAATA (SEQ ID NO:32, $MSP1_{42}$, antisense, 5334-5318, *Bam*HI). PCR primers for $MSP1_{19}$

were the same as above except *Eco*RI (sense) and *Bam*HI (antisense) restriction sites were used. Yeast two hybrid assays using these recombinant plasmids were carried out using MATCHMAKER yeast-two hybrid system 3 (CLONTECH Laboratories, Inc.), see Table 2 for summary of results.

Indirect Immunofluorescence Assay

RBCs freshly collected into citrate phosphate dextrose buffer were washed (3X) and resuspended in RPMI (20% hematocrit). Thin smears of RBCs on glass slides were allowed to air-dry and fixed in methanol (20-30 sec). The slides were washed with PBS (1X), blocked with 10% fetal bovine serum (FBS) in PBS (pH 7.4) for 1.5 h at 37 °C, and again washed with PBS (5X, 10 min each). Fixed cells were incubated with GST-$MSP1_{42}$ (1 μM), GST-$MSP1_{38}$ (8 μM), or GST (9μM) for 3.5 h at 37°C in 10% FBS. Slide samples were washed with PBS (5X), incubated with goat anti-GST antibody (1:1,000, Amersham Pharmacia Biotech), washed again (5X), and incubated with rabbit anti-goat FITC-conjugated antibody (1:60, Sigma-Aldrich, St. Louis, MO). For visualizing spectrin, fixed cells treated with 10% FBS were incubated with a rabbit antibody raised against human spectrin (Sigma-Aldrich) followed by goat anti-rabbit FITC-conjugated antibody. All dilutions of proteins and antibodies were made in 10% FBS except FITC-conjugated antibodies.

Binding $MSP1_{38}$ and $MSP1_{19}$ to RBCs in Suspension

RBCs (500 μl of 50% suspension) were treated with Nm (3 mU, *Clostridium perfringens*, Roche) in 1 ml of RPMI (37 °C, 1 h) or α-ChT (0.5 mg/ml, Sigma-Aldrich) in 1 ml RPMI (RT, 10 or 40 min) followed by PMSF (2 mM) for 30 min. To determine $MSP1_{38}$ binding, affinity purified GST-$MSP1_{38}$ was concentrated in PBS (pH 7.4) using Amicon spin column (10 kDa) and incubated with either Nm-treated or untreated human RBCs (7 μl packed volume) in PBS (pH 7.4, final 200 μl) at RT for 2 h. The mixture was passed through a bed of silicon oil (300 μl) by centrifugation. The RBC pellet was washed (1 ml) and resuspended (50-60 μl) in PBS and subjected to SDS-PAGE followed by Western blot using and anti-GST antibody (Amersham Pharmacia Biotech). GST was used as control. $MSP1_{19}$ binding: Enzyme-treated or untreated human and mouse RBCs (7 μl packed volume for human and 10 μl for

mouse RBCs) were incubated with ^{32}P-labeled $MSP1_{19}$ in PBS as above. The incubation mixture was passed through silicon oil and RBCs were washed once as above. Radioactivity associated with resulting RBCs was measured using β-scintillation counter. Negative controls included samples with no RBCs and with only RBCs (no ^{32}P-labeled $MSP1_{19}$).

Solution Binding of $MSP1_{38}$ and $MSP1_{42}$

Binding assays were performed as described (Oh, S.S., et al., *Mol Biochem Parasitol* 108:237-247, 2000) using the following conditions: 20 mM phosphate buffer (pH 7.4), 120 mM NaCl, 1 mM DTT, 1.0 mg/ml BSA, 25 °C, 3.5 h, 280 µl final vol. ^{32}P-labeled 5ABC (10, 20, 40, 80 µM) and 5BC (21, 42, 84, 168 µM) respectively bound to GST-$MSP1_{42}$ (Fig. 4B top panel) and GST-$MSP1_{38}$ (Fig. 4B middle panel) on beads in concentration-dependent manner. ^{32}P-labeled $MSP1_{19}$ bound specifically to the 5ABC domain (Fig. 4B bottom panel) as statistically analyzed by Student's *t* test. Binding to GST at comparable concentrations was not significant in all cases.

Metabolic Radiolabeling and Extraction of Parasite Proteins

Trophozoite-infected RBCs (60-100 µl packed volume) were purified on 70% Percoll gradient from a synchronized *P. falciparum* (3D7) culture and returned to culture in RPMI (without methionine, cysteine, and leucine) containing 15% human serum without adding fresh RBCs. ^{35}S-methionine and cysteine (3:1 mixture, 1.6 mCi, specific activity 1,175 Ci/mmol, NEN (PerkinElmer Life Sciences, Boston, MA) and ^{3}H-leucine (250 µCi, specific activity 166 Ci/mmol, Amersham Pharmacia Biotech) was then added and the culture was kept for 12 hr. The radioactive medium was replaced with the cold RPMI with 15% serum, and incubation continued on shaker (60-80 rpm) until segmenters and released merozoites appeared in the culture (about 8 h). Pellets were collected initially from the culture (500 rpm, 7 min) and then from the resulting supernatant (3,500 rpm, 15 min). Combined pellets were stored at –80°C or used immediately in the next step. Parasite pellets were treated with extraction buffer containing 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 5 mM EGTA, 150 mM NaCl, 0.5% Triton X-100, 0.5% BSA, 2 µg/ml aprotinin, 1 µg/ml of

leupeptin, pepstatin A, bestatin, 10 mM PMSF and protease inhibitor cocktail (1X, Roche Molecular Biochemicals, Indianapolis, IN). The mixture was kept on ice for 1hr and spun at 15,000 rpm for 15min at 4°C. The supernatant was aliquoted and either used immediately or stored at –80 °C in binding assays.

Results

Band 3-Null RBCs Are Completely Resistant to *P. falciparum* Infection

To investigate the role of Band 3 in the host RBC membrane during the *P. falciparum* invasion process, we tested the susceptibility of Band 3 null mouse RBCs to *P. falciparum* (3D7 strain) invasion *in vitro*. When infected with synchronized *P. falciparum* trophozoites, wild type and Band 3 (+/–) mouse RBCs showed a typical invasion profile after 24 hours (Figure 1A) consistent with a previously study (Klotz, F.W., et al., *J Exp Med* 165:1713-1718, 1987). However, the Band 3 null RBCs did not show any new infection (rings) by *P. falciparum*. During the course of the experiment, Band 3 (–/–) mouse RBCs remained essentially intact in the culture as judged by the smears (Figure 1B) suggesting that the increased fragility of these RBCs (Perkins, L.L., et al., *Cell* 86:917-927, 1996) could not have played a significant role in the outcome of this experiment.

We found protein 4.2 (–/–) mice (Peters, L.L., et al., *J Clin Invest* 103:1527-1537, 1999) developed parasitemia at a rate comparable to the wild type when challenged with *P. yoelii* 17XL (unpublished data). In view of these findings, the lack of Band 3 in the RBC membrane appears to be the primary cause for complete resistance to *P. falciparum* infection in our Band 3-null RBC model. We hypothesize that Band 3 is functioning as a host receptor independently or in conjunction with GPA during *P. falciparum* invasion into RBCs.

Band 3 Peptides Block *P. falciparum* Invasion into RBCs

To investigate the possible role of Band 3 as a host receptor in *P. falciparum* invasion into RBCs, we employed a peptide scanning strategy based on recent topology models of human erythroid Band 3 (Figure 2A). Synthetic peptides were derived from the putative ectodomains of human RBC Band 3, and their ability to inhibit the *P. falciparum* (3D7 strain) invasion of human RBCs in culture was tested

using both visual counting (Schlichtherle, M., et al., *Methods in Malaria Research* 2000) and ^{3}H-hypoxanthine incorporation (Chulay, J.D., et al., *Exp Parasitol* 55:138-146, 1983) methods. In the visual counting assay (Figure 2B), Band 3 peptides 5A ($p = 0.016$), 5B ($p = 0.013$), 5C ($p = 0.006$), and 6A ($p = 0.006$) showed significant levels of inhibition of invasion at 500 μM concentration as compared to other Band 3 peptides and the control (no peptide). Inhibition by these four Band 3 peptides was concentration dependent (50, 200, 500, and 1000 μM peptides, data not shown). In the ^{3}H-hypoxanthine uptake assay, peptide 6A ($p= 0.003$) showed strong inhibition whereas peptides 1 ($p= 0.148$), 5B ($p= 0.031$) and 5C ($p= 0.023$) showed moderate but significant levels of inhibition of invasion at 400 μM concentration as compared to other Band 3 peptides and control (no peptides and unrelated peptide) samples (Figure 2C). Growth inhibition study using a similar ^{3}H-hypoxanthine uptake method showed that peptides 5B ($p= 0.033$), 5C ($p= 0.032$) and 6A ($p= 0.478$) added to the culture at 400 μM concentration did not affect the intraerythrocytic growth of the parasite (Figure 2D). However, peptide 1 ($p= 0.320$), 2 ($p= 0.140$), and 5A ($p= 0.448$) samples showed mild reduction in ^{3}H-hypoxanthine uptake as compared to control (no peptide) suggesting these peptides might be inhibiting parasite growth in culture. Thus, our study showed peptides 5B, 5C and 6A were the most effective inhibitors targeting *P. falciparum* invasion among all Band 3 peptides tested. Further, invasion blocking effects of peptides 5B, 5C, and 6A did not correlate with the net charge or pI of the peptides as these properties for peptides 4B, 1, and 3A, respectively were closely similar (Figure 2E).

The IC_{50} values (50% inhibition in the parasite invasion of RBCs) determined for peptide 5C and peptide 6A using the ^{3}H-hypoxanthine incorporation method were 591 ± 131 μM and 316 ± 45 μM, respectively (mean of three experiments ± standard error). The IC_{50} values for peptides 5A and 5B could not be estimated because a saturable peptide concentration could not be reached with a low DMSO (≤1%) concentration. It is noteworthy that our peptides 5A, 5B, 5C, and 6A were designed by randomly dividing the two ectodomains of Band 3, and thus these peptides may not necessarily represent Band 3 amino acid compositions having the best inhibitory property. Our results, however, clearly demonstrate that peptides derived from two specific regions (amino acids 720-761 and 807-826) of human Band 3 inhibit the *P.*

falciparum invasion of human RBCs at a significant level and in a concentration dependent manner. These findings are consistent with the view that Band 3 functions as an important receptor in the parasite invasion of RBCs.

Band 3 Peptides Interact with *P. falciparum* Merozoite Proteins

A binding study was carried out between the synthetic Band 3 peptides and merozoite proteins to understand the mechanism by which Band 3 peptides 5B, 5C, and 6A inhibit the parasite invasion of RBCs. *P. falciparum* merozoites essentially free of contaminating RBC membrane components were isolated (Figure 3A), and total merozoite proteins were separated by SDS-PAGE (Figure 3B). The purified merozoite protein mixture did not contain human RBC ghost proteins as judged by Coomassie blue staining. This was confirmed by Western blot using anti-spectrin and anti-Band 3 antibodies (not shown). A 1:1 mixture of peptides 5C and 6A showed specific binding to a number of merozoite proteins in the binding assay using a blot overlay method (Figure 3C). Approximate molecular masses of these merozoite proteins are 175, 150, 125, 52, 48, 42, and 35 kDa (shown by arrowheads). In control samples, peptides 3A, 4A, and 2 did not show significant binding to any of these merozoite proteins, although a couple of weak signals were observed in the peptide 3A+4A sample. The peptide 5C+6A mixture did not show specific binding to RBC ghost proteins. Our blot overlay results provide evidence that Band 3 functions as a receptor in the *P. falciparum* invasion of RBCs, and suggest that the underlying mechanism for the observed inhibition of invasion involves a specific binding of the Band 3 peptides to one or more merozoite ligands, thus competitively blocking its interaction with the RBC Band 3 receptor.

Native MSP1 Binds to Recombinant Band 3

Among the merozoite proteins that specifically interacted with Band 3 peptides 5C and 6A the blot overlay binding assay (Figure 3C) were polypeptides migrating at approximately 42 kDa and 35 kDa mass. To investigate whether at least one of the two polypeptides could be the 42 kDa or 38 kDa proteolytic fragment of MSP1 ($MSP1_{42}$ or $MSP1_{38}$), recombinant human Band 3 peptide 5ABC was prepared as a GST-fusion protein and affinity purified on GSH beads (Figure 3D). *P.*

falciparum proteins metabolically labeled with ^{35}S-methionine and ^{3}H-leucine at the trophozoite stage were extracted from the mixture of late schizonts (segmenters) and naturally released merozoites using 0.5% Triton X-100. From this radiolabeled parasite protein extract, full-length MSP1 and $MSP1_{42}$ were immunoprecipitated with mAb 5.2 (MRA-94), a $MSP1_{19}$ (the 19 kDa C-terminal domain of MSP1)-specific monoclonal antibody raised against native *P. falciparum* MSP1 (Figure 3E, lane 3). In parallel, full-length MSP1 and $MSP1_{42}$ in the total protein mixture also bound specifically to the 5ABC domain in the binding assay using GST-5ABC (lane 1) and GST (lane 2, control) beads. Similar to the blot overlay assay results, a few radioactive protein Bands were specifically associated with GST-5ABC beads (not shown).

Characterization of MSP1-Band 3 Interaction

During its maturation, *P. falciparum* MSP1 (full-length) is processed to give proteolytic fragments referred to as $MSP1_{83}$, $MSP1_{30}$, $MSP1_{38}$, and $MSP1_{42}$ which together form a non-covalent complex on the merozoite surface as merozoites are released into circulation from infected RBCs (Holder, A.A., et al., *Mem Inst Oswaldo Cruz* 3:37-42, 1992). $MSP1_{42}$ then undergoes secondary processing producing $MSP1_{19}$ (C-terminal domain of $MSP1_{42}$) that is retained on the merozoite surface and carried into the newly invaded RBC while all other MSP1 fragments are shed off by an unclear mechanism. To characterize the interaction of the Band 3 receptor with proteolytic fragments of MSP1, two independent binding studies were carried out in solution *in vitro* and in yeast two-hybrid system *in vivo*. For the *in vitro* binding study, *P. falciparum* $MSP1_{38}$, $MSP1_{42}$ and $MSP1_{19}$ (Figure 4A) as well as human Band 3 peptides 5BC and 5ABC (Figure 3D) were expressed in *E. coli* and purified as GST-fusion proteins. GST-$MSP1_{38}$ was purified as a mixture of three C-terminal truncated polypeptides. GST-$MSP1_{38}$, GST-$MSP1_{42}$ and GST-$MSP1_{19}$ reacted with *P. falciparum* MSP1 T9/94 rabbit antiserum (MRA-75) generated against full-length MSP1 in Western blot (not shown). Similarly, GST-$MSP1_{42}$ and GST-$MSP1_{19}$ reacted with mAb 5.2 (Western blot shown for GST-$MSP1_{19}$ in Figure 4A, lane 9). Further, GST-$MSP1_{19}$, GST-5BC, and GST-5ABC were labeled with ^{32}P and treated

with thrombin to afford pure ^{32}P-labeled $MSP1_{19}$ (Figure 4A, lane 10), 5BC (lane 11), and 5ABC (lane 12).

In solution-binding assay, ^{32}P-labeled 5ABC and 5BC bound to GST-$MSP1_{42}$ (K_d= 36 μM) and GST-$MSP1_{38}$ (K_d= 67 μM), respectively, when neither showed significant binding to GST alone (Figures 4B, 4C, and 4D). Further, ^{32}P-labeled $MSP1_{19}$ bound to GST-5ABC at a significant level (p= 0.027) as compared to the GST control sample. These results demonstrate specific binding interactions between the MSP1 domain and the Band 3 peptide domain. The three-dimensional structure for the 5ABC or 5BC region of Band 3 is not known. A difference of 11 amino acids in the primary structure of 5ABC and 5BC may be important for showing different binding property towards MSP1 in our *in vitro* experiments. What is clearly implicated from these results is that an ectoplasmic region of human Band 3 represented by amino acids 720-761 constitutes the binding site for $MSP1_{38}$, $MSP1_{42}$, and $MSP1_{19}$ presumably involving a number of specific binding interactions contained within this region.

In the second approach using a GAL4-based yeast two-hybrid assay, Band 3 peptides (5ABC, 5BC, 6AB) and MSP1 domains ($MSP1_{38a}$, $MSP1_{38b}$, $MSP1_{42}$, $MSP1_{19}$) were expressed as a fusion to the GAL4 DNA-binding (DNA-BD) domain and GAL4 activation domain (AD), respectively. $MSP1_{38}$ was divided into $MSP1_{38a}$ and $MSP1_{38b}$ in view of C-terminal truncated $MSP1_{38}$ used in the solution-binding assay. (See Table 2). Peptide 5ABC interacted with $MSP1_{42}$, $MSP1_{19}$, and $MSP1_{38b}$, peptide 5BC interacted with only $MSP1_{38a}$, and peptide 6AB interacted with $MSP1_{42}$ and $MSP1_{19}$. For the yeast two-hybrid assay, the co-transformation method was used to analyze the protein interaction in yeast AH109 cells using SD/–Leu/–Trp selection plates. Activation of the *MEL1* reporter gene upon specific binding of a MSP1 domain to a Band 3 peptide gave positive blue colonies using α-galactosidase assay. Plasmids expressing only the inserted gene of a Band 3 peptide or MSP1 domain did not undergo autonomous transcriptional activation of the reporter gene. No interaction was observed in other two-hybrid samples of Band 3 peptide and MSP1 domain. All positive and negative controls gave anticipated results. Specific interactions demonstrated with peptides 5ABC and 5BC in the yeast two-hybrid assay were consistent with the solution-binding assay results (Figures 4B, 4C, and 4D). The

expression of 6AB as a soluble form in yeast cells (data not shown) provides support that the observed 6AB-$MSP1_{42}$ and 6AB-$MSP1_{19}$ interactions are specific. Binding results from two independent *in vitro* and *in vivo* methods summarized in Figure 4E were remarkably similar indicating that a key function of $MSP1_{38}$, $MSP1_{42}$, and $MSP1_{19}$ is to interact with Band 3 in the RBC membrane during merozoite invasion of RBCs.

Table 2. Summary of Yeast Two-Hybrid Assay

Fusion to DNA-BD (pGBKT7 vector)	Fusion to AD (PgaDT7 vector)	Binding Property
None	None	–
p53	None	–
None	SV40 large T-antigen	–
p53	SV40 large T-antigen	+++
Lamin C	SV40 large T-antigen	–
5ABC	SV40 large T-antigen	–
5BC	SV40 large T-antigen	–
6AB	SV40 large T-antigen	–
Lamin C	$MSP1_{38a}$	–
Lamin C	$MSP1_{38b}$	–
Lamin C	$MSP1_{42}$	–
p53	$MSP1_{42}$	–
p53	$MSP1_{19}$	–
5ABC	$MSP1_{38a}$	–
5ABC	$MSP1_{38b}$	+
5ABC	$MSP1_{42}$	+++
5ABC	$MSP1_{19}$	+++
5BC	$MSP1_{38a}$	++
5BC	$MSP1_{38b}$	–
5BC	$MSP1_{42}$	–

5BC	$MSP1_{19}$	–
6AB	$MSP1_{38a}$	–
6AB	$MSP1_{38b}$	–
6AB	$MSP1_{42}$	+++
6AB	$MSP1_{19}$	+++

Proteolytic Fragments of MSP1 Bind to RBCs in Sialic Acid-Independent Manner

Earlier studies showed that native *P. falciparum* MSP1 (full-length) bound to RBCs in sialic acid-dependent manner (Perkins, M.E., et al., *J Immunol* 141:3190-3196, 1988; Su, S., et al., *J Immunol* 151:2309-2317, 1993). More recently, however, it has been shown that a number of peptides derived from $MSP1_{83}$, $MSP1_{38}$, and $MSP1_{42}$ bound to sialic acid-depleted RBCs with relatively high affinity (Urquiza, M., et al., *Parasite Immunol* 18:515-526, 1996). A recombinant segment (115 amino acids) of $MSP1_{38}$ referred to as p115MSP-1 also bound to wild-type human RBCs as well as En(a–) human RBCs lacking GPA (Nikodem, D., et al., *Mol Biochem Parasitol* 108:79-91, 2000).

To further examine the RBC binding property of MSP1 proteolytic fragments, we first carried out a binding study using GST-$MSP1_{38}$ and intact human RBCs either untreated or pretreated with neuraminidase (Nm). Removal of sialic acids on the surface of Nm-treated RBCs was confirmed by periodic acid-Schiff (PAS) staining using RBC ghosts (Figure 5A, lane 9). Both types of RBCs were incubated with purified GST-$MSP1_{38}$, sedimented through a bed of silicon oil as described (Nikodem, D., et al., *Mol Biochem Parasitol* 108:79-91, 2000) and subjected to SDS-PAGE followed by Western blotting using anti-GST antibody. Two truncated forms of GST-$MSP1_{38}$ (45 and 30 kDa Bands shown above in Figure 4A, lane 5) bound to both treated (Figure 5B, lanes 1) and untreated (lane 3) intact RBCs in suspension. GST alone did not bind to either type of RBCs (lanes 2, 4). These results demonstrate that $MSP1_{38}$ specifically interacted with the extracellular component of human RBCs in sialic acid-independent manner. This is consistent with our finding that peptide 5BC representing a non-glycosylated ectodomain of Band 3 bound to $MSP1_{38}$ in solution and $MSP1_{38a}$ in the yeast two-hybrid system.

In demonstrating the sialic acid-independent binding of $MSP1_{42}$ to intact RBCs, we used its C-terminal secondary processing fragment $MSP1_{19}$ known to be carried into newly invaded RBCs (Blackman, M.J., et al., *J Exp Med* 172:379-382, 1990). To carry out this study, we treated intact human and mouse RBCs with either chymotrypsin (ChT) or Nm (Figure 5A). In ChT-treated human (Figure 5A, lane 3) and mouse (lane 6) RBCs, full-length Band 3 (arrowheads) was digested into 55 kDa N-terminal and 38 kDa C-terminal fragments (arrows) as reported (Steck, T.L., et al., *Biochemistry* 17:1216-1222, 1978). The 38 kDa fragment known to be less stable than the 55 kDa fragment often appeared as a diffused faint Band in Coomassie gel (lane 6). As expected, Band 3 (arrowhead) was intact in Nm-treated human RBCs (lane 2) and mouse RBCs (lane 5). Western blotting of the human RBC ghost samples using anti-Band 3 antibody specific for the N-terminal cytoplasmic domain of human Band 3 confirmed these results (lanes 10-12). The extracellular region of GPA was also digested by ChT at an appreciable rate as evident in the PAS-stained gel (lane 8) and anti-GPA Western blot (lane 14) of human RBC samples. A significant level of sialic acid residues attached to ChT-digested GPA fragments was retained on the RBC surface as judged by PAS staining (land 8). The ChT-digested GPA fragments at approximately 62 kDa and 47 kDa molecular mass (shown with asterisks) in the human RBC sample (lane 14) were consistent with the previous report (Roggwiller, E., et al., *Mol Biochem Parasitol* 82:13-24, 1996).

These various types of RBCs were reacted with ^{32}P-labeled $MSP1_{19}$ (Figure 4A, lane 10) in suspension, and the radioactivity associated with RBCs was analyzed. Human RBCs treated with ChT for 10 min and 40 min showed 30% (p= 0.010) and 37% (p= 0.040) reduction and with Nm (40 min) showed 52% increase (p= 0.054) in their ability to bind $MSP1_{19}$ as compared to the untreated RBCs (Figure 5C). In mouse RBC samples, ChT-treated RBCs (40 min) showed 41% reduction (p= 0.005) and Nm-treated RBCs (40 min) showed 51% increase (p= 0.086) as compared to the untreated RBCs. These results show for the first time that *P. falciparum* $MSP1_{19}$ binds to intact human and mouse RBCs in sialic acid-independent manner. Previously, a synthetic peptide referred to as peptide 5501 (20 amino acids) derived

from the N-terminus of $MSP1_{19}$ was reported to bind human RBCs (Urquiza, M., et al., *Parasite Immunol* 18:515-526, 1996). Further, the reduced binding of $MSP1_{19}$ in ChT-treated RBC samples in our assays is directly correlated with alterations in Band 3 and GPA peptide backbones on the RBC surface. Nm treatment provided both human and mouse RBCs with increased ability to bind $MSP1_{19}$ demonstrating that the binding interaction does not involve sialic acids. Presumably, the increase in binding results from relatively unhindered access to the protein receptor upon removal of sialic acid residues from the RBC surface.

Band 3 is important for *P. falciparum* MSP1 Binding to RBCs

Two approaches were considered to obtain evidence that Band 3 mediates sialic acid-independent interaction between MSP1 and RBCs. First, the above-described RBC binding assay was performed using ^{32}P-labeled $MSP1_{19}$ and intact Band 3 (–/–) mouse RBCs in suspension. As compared to untreated wild-type mouse RBCs, there was a 72% reduction ($p = 0.277 \times 10^{-6}$) of radioactivity associated with Band 3 (–/–) mouse RBCs (Figure 5C). However, since background radioactivity from the negative control sample containing no RBCs was about 18% of the positive control (untreated wild-type RBCs), the actual radioactivity associated with Band 3 (–/–) mouse RBCs was mere 10% above the background. Thus, intact Band 3 (–/–) mouse RBCs lacking both Band 3 and GPA from the plasma membrane showed a relatively insignificant level of binding to $MSP1_{19}$. In the second method, we carried out an indirect immunofluorescence assay (IFA) using wild-type human and mouse RBCs and Band 3 (–/–) mouse RBCs fixed in methanol. GST-$MSP1_{42}$ and GST-$MSP1_{38}$ (truncated forms) bound to human as well as mouse wild-type RBCs while GST alone did not, demonstrating that the observed binding was specific to the $MSP1_{42}$ and $MSP1_{38}$ domain, respectively. However, neither GST-$MSP1_{42}$, GST-$MSP1_{38}$, nor GST alone bound to Band 3 (–/–) mouse RBCs. In the indirect immunofluorescence assay of *P. falciparum* MSP1 binding to human and mouse RBCs, Anti-spectrin antibody staining confirmed all RBCs were morphologically normal.

Our truncated $MSP1_{38}$ (the 45 kDa GST-fusion protein) specifically bound to sialic acid-depleted RBCs (Figure 5B), and the p115MSP-1 construct substantially overlapping (at least about 60 amino acids) our $MSP1_{38}$ was shown to bind to intact human RBCs lacking the sialoglycoprotein GPA (Nikodem, D., et al., *Mol Biochem Parasitol* 108:79-91, 2000). In view of these findings, our IFA results demonstrate that the peptide backbone of Band 3 in the RBC membrane is important for binding $MSP1_{38}$ to the RBC surface. In this context, peptide 5ABC representing a putative ectoplasmic region (amino acids 720-761) of Band 3 specifically bound to $MSP1_{38}$ and $MSP1_{38a}$ in our binding assays (Figure 4E). This region of Band 3 shares 98% sequence identity between mouse and human. Our results thus indicate that *P. falciparum* $MSP1_{38}$ interacts with the Band 3 receptor on both human and mouse RBC surface during the parasite invasion of RBCs.

The $MSP1_{42}$ binding results in our IFA are consistent with the binding of its C-terminal fragment $MSP1_{19}$ to RBCs in suspension (Figure 5C). Further, the level of $MSP1_{19}$ binding to RBCs decreased considerably with the limited ChT digestion of Band 3 and GPA peptide backbones on the RBC surface. Previously, ChT-treated human RBCs were shown to have marked reduction in invasion by *P. falciparum* as compared to untreated RBCs (Perkins, M., *J Cell Biol* 90:563-567, 1981). Since recombinant *P. falciparum* $MSP1_{42}$ and $MSP1_{19}$ bound to two distinct non-glycosylated ectoplasmic regions (5ABC and 6AB) of erythroid Band 3 (Figure 4E) and native $MSP1_{42}$ specifically bound to 5ABC (Figure 3E), our results taken together show that Band 3 functions as the receptor also for $MSP1_{42}$ and $MSP1_{19}$. A possibility that the peptide backbone of GPA might also play a secondary role in binding $MSP1_{42}$ and/or $MSP1_{19}$ to RBCs cannot be completely ruled out. Results from our RBC binding studies support the idea that Band 3 (–/–) RBCs are completely refractory to *P. falciparum* invasion due to the lack of an important interaction involving host Band 3 and proteolytic fragments of merozoite MSP1 such as $MSP1_{38}$, $MSP1_{42}$ and $MSP1_{19}$.

Example 6 Identification of *Plasmodium* Polypeptides that Interact with Band 3

Methods

The "Bait" construct

The bait construct containing the 5ABC domain (amino acids 720-761) of human Band 3 was prepared by a PCR amplification of the corresponding gene from a human reticulocyte cDNA library and cloning into a yeast two-hybrid vector pGBKT7 (Clontech) as a fusion to GAL4 DNA-binding (DNA-BD). Primers used were 5'-CCGGAATTCGGGATGCCCTGGCTCAGTGCCA-3'(SEQ ID NO:36, sense, 2272-2293, EcoRI), 5'-CCGGGATCCTTAGATCCGCTGCTCTTTGACCTC-3' (SEQ ID NO:37. antisense, 2397-2377, BamHI). The bait construct pGBKT7-5ABC was transformed into yeast AH109. The expression of 5ABC domain as a soluble fusion protein in yeast was confirmed by Western blotting of the cell lysate supernatant as described in the Clontech manual. The absence of autonomous transcriptional activation of the reporter gene by the bait domain 5ABC was confirmed on agar plates made with a minimal synthetic dropout medium (SD) lacking tryptophan (SD/-Trp), tryptophan and histidine (SD/-Trp-His), and tryptophan and adenine (SD/-Trp-Ade) using the X-α-Gal assay according to the Clontech manual.

Screening of the cDNA library in yeast two-hybrid system

The screening of *P. falciparum* (3D7 strain) cDNA library transformed into yeast PJ69-2A (Clontech) was performed by the standard yeast mating method using the bait construct pGBKT7-5ABC transformed into yeast Y187 (Clontech) as described in the Clontech manual (MATCHMAKER Two-Hybrid System 3). The mating mixture was spread onto 50 large (150 mm) plates of SD/-His-Leu-Trp (TDO). The cells were grown at 30°C for 10 days. His^+ colonies selected by this procedure were streaked onto SD/-His-Ade-Leu-Trp/X-a-Gal (QDO) plates and grown for 1 week at 30°C. Both positive (pGBKT7-53 + pGADT7-T) and negative (pGBKT7 + pGADT7) controls (Clontech) were included at each round of selection. Ade^+, His^+, Mel^+ yeast colonies were selected from these plates for further analysis. Following overnight growth in the SD/-Leu-Trp-His broth at 30 °C, cells from each clone were harvested. Plasmid DNAs isolated upon cell lysis were transformed into

E. coli DH5α, and transformants were selected on LB/ampicillin plates. A selected colony from each transformation plate was subcultured overnight in the LB/ampicillin broth and the plasmid DNA was isolated by alkaline lysis. The nucleotide sequence of *P. falciparum* cDNA inserts was determined to identify these positive clones.

Results

Analysis of positive clones

From the cDNA library containing 9.8 X 10^6 independent transformants, we selected 116 His^+ colonies on SD/-His-Leu-Trp plates. These 116 His^+ colonies were subjected to a second round of selection by streaking onto the SD/-Ade-His-Trp-Leu plate. Because the induction of the more tightly controlled GAL2-ADE2 reporter gene is required under these higher stringent selection conditions (Ade^+), only 20 out of 116 colonies grew. Approximately 500 base pair sequence of *P. falciparum* cDNA inserts was determined from the 5' end of the gene. Subsequent Blast analyses (at the NCBI and PlasmoDB website) of these insert cDNA sequences revealed that two of them were *P. falciparum* ABRA and RhopH3 (Table 3). All other cDNA insert sequences (total= 18) were found only in PlasmoDB. Upon further analysis of the insert sequences, we found that only six of the eighteen insert cDNAs were non-redundant and in correct reading frame. These six were novel *P. falciparum* genes with no known functions associated with their gene products (Table 3). We have designated names to these six gene products as Band 3 Binding Protein (BBP)-1, 2, 3, 4, 5, and 6. The binding interaction between the Band 3 peptide 5ABC and each of the eight *P. falciparum* gene products was independently confirmed using the cotransformation and/or mating method (Clontech manual) in subsequent yeast two-hybrid assays under various stringency conditions as summarized in Table 3.

Table 3. Summary of cDNA library screening in yeast two-hybrid system

	BD-5ABC + AD-X [a]	-T- L	-T-L- H	-T-L- A	-T-L-A- H	Gene Name used by PlasmoDB [b]	Designated Name [c]
Clone Number	8	++	++	+	+	chr4_1.gen_205	BBP-1
	12	++	++	+	+	chr9_1.gen_311 [d]	RhopH3
	14	++	++	+	+	chr12_1.gen_395 [e]	ABRA
	48	++	++	+	+	chrBLOB_004238.gen_2	BBP-2
	59	++	++	+	+	chr5_1.gen_122	BBP-3
	74	++	++	+	+	chr14_1.gen_490	BBP-4
	94	++	++	+	+	chr7_000072.gen_1	BBP-5
	101	++	++	+	+	chr5_1.gen_79	BBP-6

[a] X denotes the clone number used in our cDNA library screening assay.

[b] See attachment, Supplemental Material for Dataset 2: Description of genes by PlasmoDB.

[c] BBP (Band 3 Binding Protein)-1, 2, 3,4, 5, and 6 are names we have designated to respective gene products.

[d] *P. falciparum* RhopH3, GenBank Accession No: M65059.

[e] The 101 KDa *P. falciparum* malaria antigen (p101) termed Acidic Basic Repeat Antigen (ABRA), GenBank Accession No: J03902.

Table 4 depicts the Blast Sequence Homology results obtained for the Band 3 peptides having SEQ ID NOs:1, 2, 3, and 4.

TABLE 4. BAND 3 BLAST HOMOLOGY SEQUENCES

SEQ ID NO. 1
P23562,NP_036783.1,CAA31128.1,NP_000333.1,CAA27555.1,NP_035533.1,AAA37278.1,AAD43354.1,AAD43593.1,A30816 P15575,
P32847,AAF19584.2,AAG23156.1,AAG23157.1,AAG23155.1,AAF00977.1,NP_003031.1,AAF23240.1,XP_004678.1,NP_033233.1,AAG23158.1,AAC50964.1,NP_058744.1,AAF19583.2,AAB66833.1,AAC59881.1,AAG23154.1,P48746,O18917,NP_033234.1,AAG25582.1,AAB05850.1,AAG25583.1,XP_002605.1,NP_058745.1,AAD14330.1,NP_005061.1,S31828,CAA60670.1,Q9Z0S8,AAF50207.1,BAA34459.1,NP_067505.1,NP_004849.1,NP_071341.1,BAB17922.1,AAF50207.1,AAF52496.1,AAF52497.1,KADBID 004606,
AAA54840.1,AAA54837.1,AAA54839.1,NM_012651.1,X77738.1,NM_000342.1,M27819.1,L35930.1,X12609.1,J02756.1,X03917.1,M29379.1,NM_011403.1,X02677.1,J04793.1,XM_008364.1,AC003043.1,AF163826.1,AF163828.1,AF163827.1,M19496.1,M23404.1,X61699.1,Z50848.1,U62531.1,U76669.2,AF012895.1,AF255774.1,NM_009207.1,J04036.1,XM_004678.1,NM_003040.1,S45791.1,X62137.1,NM_017048.1,U48889.1,J05166.1,X03918.1,U20523.1,AF120099.1,M87060.1,AC009955.4,NM_009208.1,M28383.1,AF031650.1,NM_017049.1,AF294651.1,J05167.1,S80168.1,L27213.1,XM_002605.1,NM_005070.1,U05596.1,X87211.1,X70797.1,AF121253.1,AE003550.2,AE003550.2,BF760317.1,BF724738.1,BF726058.1,BF726058.1,BF726058.1,AW239627.1,BE255812.1,BE667859.1,BE683882.1,BE259443.1,BF760317.1,BF724738.1,BE683941.1,AA822979.1,BE512723.1,BF726058.1,AL121219.1,BE231685.1,AA362927.1,BF526005.1,F06947.1,AI592399.1,AI121401.1,BE683881.1,BF688963.1,BF688491.1,AW372960.1,AA755536.1,N58147.1,AW358179.1,BE387636.1,AA979500.1,T86708.1,AC025326.3,AC010973.4,AC016330.5,AC016170.2,AC010044.5,AC014376.1,AL291529.1,I08446.1,E15207.1,I08447.1,AX001285.1,AX001281.1,AX001279.1,NM_000342.1,XM_008364.1,NM_003040.1,XM_004678.1,XM_002605.1,NM_005070.1,NP_000333.1,NP_003031.1,XP_004678.1,XP_002605.1,NP_005061.1,NT_010755.1,

Seq ID NO:2
CAA31128.1, NP_000333.1, P23562, NP_036783.1, CAA27555.1, NP_035533.1, AAA37278.1, AAD43354.1, AAD43593.1, AAF19584.2, AAG23156.1, AAG23157.1, AAG23155.1, Q9Z0S8, AAF00977.1, NP_003031.1, AAF23240.1, XP_004678.1, NP_033233.1, AAG23158.1, AAC50964.1, NP_058744.1, AAF19583.2, AAB66833.1, , AAC59881.1, AAG23154.1, P48746, AAD14330.1, , P15575, AAA48, AAB23405.1, , P32847, AAF50207.1, AAA54840.1, AAA54837.1, AAA54839.1, X77738.1, NM_000342.1, AC003043.1, M27819.1, L35930.1, X12609.1, XM_008364.1, J02756.1, NM_012651.1, X03917.1, NM_011403.1, X02677.1, M29379.1, J04793.1, AF163826.1, AF163828.1, AF163827.1, U20523.1, X03918.1, U62531.1, U76669.2, AF012895.1, AF120099.1, AF121253.1, NM_009207.1, J04036.1, XM_004678.1, AF255774.1, NM_003040.1, S45791.1, X62137.1, U48889.1, NM_017048.1, J05166.1, S80168.1, M19496.1, M23404.1, BF760317.1, BF724738.1, BF724738.1,

BF724738.1, AL361787.1, BE512723.1, AA822979.1, BE255812.1, AL121219.1, BE667859.1, BE259443.1, BF760317.1, BE868202.1, AI121401.1, BF724738.1, BE231685.1, AW446011.1, AA362927.1, BF526005.1, I08446.1, E15207.1, I08447.1, AC025326.3, AC010973.4, NP_000333.1, NM_000342.1, XM_008364.1, NT_010755.1,

SEQ ID NO:3
', CAA31128.1, NP_000333.1, P23562, NP_036783.1, CAA27555.1, NP_035533.1, AAA37278.1, AAD43354.1, AAD43593.1, AAA54840.1, AAA54837.1, AAA54839.1, X77738.1, NM_000342.1, M27819.1, AC003043.1, L35930.1, X12609.1, XM_008364.1, NM_012651.1, J02756.1, M29379.1, NM_011403.1, X03917.1, X02677.1, J04793.1, AF163826.1, AF163828.1, AF163827.1, AC025326.3, I08446.1, E15207.1, I08447.1, NM_000342.1, XM_008364.1, NP_000333.1, NT_010755.1,

SEQ ID NO:4
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EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

All references disclosed herein are incorporated by reference in their entirety.

We claim:

Claims

1. An isolated Band 3 polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO. 1, 2, 3, and 4 as shown herein:

SEQ ID NO:1: GMPWLSATTVRSVTHANALT (also referred to herein as sequence $B3_{5A}$);
SEQ ID NO:2: SVTHANALTVMGKASTPGAA (also referred to herein as sequence $B3_{5B}$);
SEQ ID NO:3: GKASTPGAAAQIQEVKEQRI (also referred to herein as sequence $B3_{5C}$);
SEQ ID NO:4: DRILLLFKPPKYHPDVPYVK (also referred to herein as sequence $B3_{6A}$); and unique fragments thereof, wherein the unique fragments

(1) bind to an MSP-1 polypeptide and

(2) exclude the sequences set forth in Table 4:Band 3 Blast Homology Sequences.

2. An isolated nucleic acid molecule that encodes the isolated polypeptide of claim 1.

3. An expression vector comprising the isolated nucleic acid of claim 2 operably linked to a promoter.

4. A host cell transfected or transformed with an expression vector of claim 3.

5. An immunogenic composition comprising:
one or more isolated polypeptides of claim 1; and
a pharmaceutically acceptable carrier;
wherein the polypeptides are present in an effective amount to induce an immune system response.

6. The composition of claim 5, further comprising an adjuvant.

7. A method of making a medicament, comprising:

placing one or more isolated polypeptides of claim 1 in a pharmaceutically acceptable carrier.

8. A method for identifying a candidate mimetic of an isolated polypeptide of claim 1, comprising

providing an MSP-1 polypeptide which binds the isolated polypeptide of claim 1,

contacting the MSP-1 polypeptide with a test molecule, and

determining the binding of the test molecule to the MSP-1 polypeptide, wherein a test molecule which binds to the MSP-1 polypeptide and inhibits binding of the MSP-1 polypeptide to the polypeptide of claim 1 is a candidate mimetic of the isolated polypeptide of claim 1.

9. A protein microarray comprising at least one isolated Band 3 polypeptide selected from the group consisting of SEQ ID NOS. 1, 2, 3, and 4.

10. An anti-Band 3 antibody or fragment thereof that selectively binds to a polypeptide of claim 1, wherein the antibody inhibits infection of cells by *P. falciparum* merozoite malaria parasite.

11. An anti-idiotype antibody which selectively binds to the idiotype of the antibody of claim 10.

12. A method for making an anti-idiotypic antibody comprising:

immunizing an animal with an antibody of claim 10 under conditions to elicit an immune system response to an idiotype of said antibody of claim 10.

13. A method for treating a malaria infection, comprising:

administering to a subject in need of such treatment, an effective amount of an anti-Band 3 antibody of claim 10 to treat the malaria infection.

14. A method for inducing an immune system response to treat a malaria infection, comprising:

administering to a subject in need of such treatment, an effective amount of an anti-Band 3 antibody of claim 10 under conditions to induce an anti-idiotypic immune response to the anti-Band 3 antibody idiotype.

15. A method for identifying a candidate mimetic of a MSP-1 polypeptide, comprising

providing an isolated Band 3 polypeptide which binds a MSP-1 polypeptide,

contacting the Band 3 polypeptide with a test molecule, and

determining the binding of the test molecule to the Band 3 polypeptide,

wherein a test molecule which binds to the isolated Band 3 polypeptide and inhibits binding of the Band 3 polypeptide to the MSP-1 polypeptide is a candidate mimetic of the MSP-1 polypeptide.

16. The method of claim 15, wherein the MSP-1 polypeptide has a sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:33, SEQ ID NO. 34, and SEQ ID NO:35.

17. The method of claim 15, wherein the test molecule is an antibody.

18. An isolated polypeptide, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs:11, 12, 13, 33, 34, and 35, or fragments thereof.

19. A pharmaceutical composition comprising:

one or more isolated polypeptides of claim 18 and

a pharmaceutically acceptable carrier;

wherein the polypeptides are present in an effective amount to induce an immune system response.

20. The pharmaceutical composition of claim 19, further comprising an adjuvant.

21. A method of making a medicament, comprising:

placing one or more isolated polypeptides of claim 19 in a pharmaceutically acceptable carrier.

22. A method of preventing or treating a malaria infection, comprising administering a pharmaceutical composition of claim 19 to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection.

23. A malaria polypeptide binding polypeptide that selectively binds to a isolated malaria polypeptide of claim 18, wherein the binding polypeptide is an antibody or antigen-binding fragment of an antibody.

24. A pharmaceutical composition comprising the malaria polypeptide binding polypeptide of claim 23, in a pharmaceutically acceptable carrier.

25. A method of preventing or treating a malaria infection, comprising administering the pharmaceutical composition of claim 24 to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection.

26. An isolated nucleic acid, wherein the nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs:54-59, or fragments thereof.

27. An isolated Band 3 polypeptide, comprising an amino acid sequence selected from the group consisting of SEQ ID NO. 1, 2, 3, and 4 as shown herein:

SEQ ID NO:1: GMPWLSATTVRSVTHANALT (also referred to herein as sequence $B3_{5A}$);

SEQ ID NO:2: SVTHANALTVMGKASTPGAA (also referred to herein as sequence $B3_{5B}$);

SEQ ID NO:3: GKASTPGAAAQIQEVKEQRI (also referred to herein as sequence $B3_{5C}$);

SEQ ID NO:4: DRILLLFKPPKYHPDVPYVK (also referred to herein as sequence $B3_{6A}$), and unique fragments thereof, wherein the unique fragments

(1) bind to an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53, or fragment thereof, and

(2) exclude the sequences set forth in Table 4:Band 3 Blast Homology Sequences.

28. An isolated nucleic acid molecule that encodes the isolated Band 3 polypeptide of claim 27.

29. An expression vector comprising the isolated nucleic acid of claim 29 operably linked to a promoter.

30. A host cell transfected or transformed with an expression vector of claim 29.

31. An immunogenic composition comprising:

one or more isolated Band 3 polypeptides of claim 27 and

a pharmaceutically acceptable carrier;

wherein the Band 3 polypeptides are present in an effective amount to induce an immune system response.

32. The composition of claim 31, further comprising an adjuvant.

33. A method of making a medicament, comprising:

placing one or more isolated Band 3 polypeptides of claim 27 in a pharmaceutically acceptable carrier.

34. A method for identifying a candidate mimetic of an isolated Band 3 polypeptide of claim 27, comprising

providing a malaria polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53, or fragment thereof that binds the isolated Band 3 polypeptide or fragment thereof of claim 27,

contacting the malaria polypeptide or fragment thereof, with a test molecule, and

determining the binding of the test molecule to the malaria polypeptide or fragment thereof, wherein a test molecule which binds to the polypeptide or fragment thereof and inhibits binding of the isolated Band 3 polypeptide to the malaria polypeptide, is a candidate mimetic of the isolated Band 3 polypeptide of claim 27.

35. A method for identifying a candidate mimetic of an isolated malaria polypeptide, comprising

providing a Band 3 molecule which binds a malaria polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs:46-53,

contacting the Band 3 molecule with a test molecule, and

determining the binding of the test molecule to the Band 3 molecule, wherein a test molecule which binds to the Band 3 molecule and inhibits binding of the malaria polypeptide with the Band 3 polypeptide is a candidate mimetic of the malaria polypeptide.

36. The method of claim 35, wherein the test molecule is an antibody.

37. An isolated polypeptide molecule comprising an amino acid sequence selected from the group consisting SEQ ID NOs:46-52.

38. A pharmaceutical composition comprising:

one or more isolated polypeptides of claim 37 and

a pharmaceutically acceptable carrier;

wherein the polypeptides are present in an effective amount to induce an immune system response.

39. The pharmaceutical composition of claim 38, further comprising an adjuvant.

40. A method of making a medicament, comprising:

placing one or more isolated polypeptides of claim 38 in a pharmaceutically acceptable carrier.

41. A method of preventing or treating a malaria infection, comprising administering a pharmaceutical composition of claim 38 to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection.

42. A malaria polypeptide binding polypeptide that selectively binds to a isolated malaria polypeptide of claim 37, wherein the binding polypeptide is an antibody or antigen-binding fragment of an antibody.

43. A pharmaceutical composition comprising the malaria polypeptide binding polypeptide of claim 42, in a pharmaceutically acceptable carrier.

44. A method of preventing or treating a malaria infection, comprising administering the pharmaceutical composition of claim 43 to a subject in need of such treatment in an amount effective to prevent or treat the malaria infection.

45. An isolated nucleic acid molecule selected from the group consisting of:

(a) nucleic acid molecules which hybridize under stringent conditions to a nucleic acid molecule comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:38-44 and which codes for a *Plasmodium* polypeptide,

(b) deletions, additions and substitutions of the nucleic acid molecules of (a), which code for a *Plasmodium* polypeptide,

(c) nucleic acid molecules that differ from the nucleic acid molecules of (a) or (b) in codon sequence due to the degeneracy of the genetic code, and

(d) complements of (a), (b) or (c).

46. The isolated nucleic acid molecule of claim 45, wherein the isolated nucleic acid molecule comprises a nucleotide sequence selected from the group consisting of SEQ ID NO:38-44.

47. An isolated nucleic acid molecule selected from the group consisting of:

(a) a unique fragment of the nucleotide sequence selected from the group consisting of:

nucleotides 1-1287 of SEQ ID NO:38 between 12 and 1286 nucleotides in length,

nucleotides 1-3576 of SEQ ID NO:39 between 12 and 3557 nucleotides in length,

nucleotides 1-903 of SEQ ID NO:40 between 12 and 902 nucleotides in length,

nucleotides 1-1203 of SEQ ID NO:41 between 12 and 1202 nucleotides in length,

nucleotides 1-3996 of SEQ ID NO:42 between 12 and 3995 nucleotides in length, and

nucleotides 1-876 of SEQ ID NO:43 between 12 and 875 nucleotides in length, and

nucleotides 1-2712 of SEQ ID NO:44 between 12 and 2711 nucleotides in length, and

(b) complements of (a),

wherein the unique fragments exclude nucleic acids having nucleotide sequences that are contained within SEQ ID NO:38-44, and that are known as of the filing date of this application.

48. An expression vector comprising the isolated nucleic acid molecule of claim 46 operably linked to a promoter.

49. An isolated polypeptide molecule comprising a unique fragment of amino acid sequence SEQ ID NO:53 that binds to a Band 3 polypeptide.

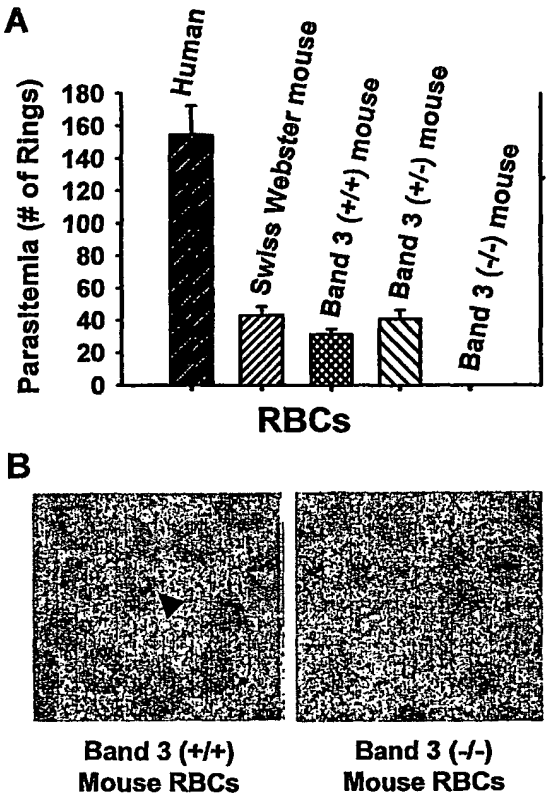
A
Parasitemia (# of Rings)
180
160
140
120
100
80
60
40
20
0
Human
Swiss Webster mouse
Band 3 (+/+) mouse
Band 3 (+/-) mouse
Band 3 (-/-) mouse
RBCs
B
Band 3 (+/+) Mouse RBCs
Band 3 (-/-) Mouse RBCs

Figure 1

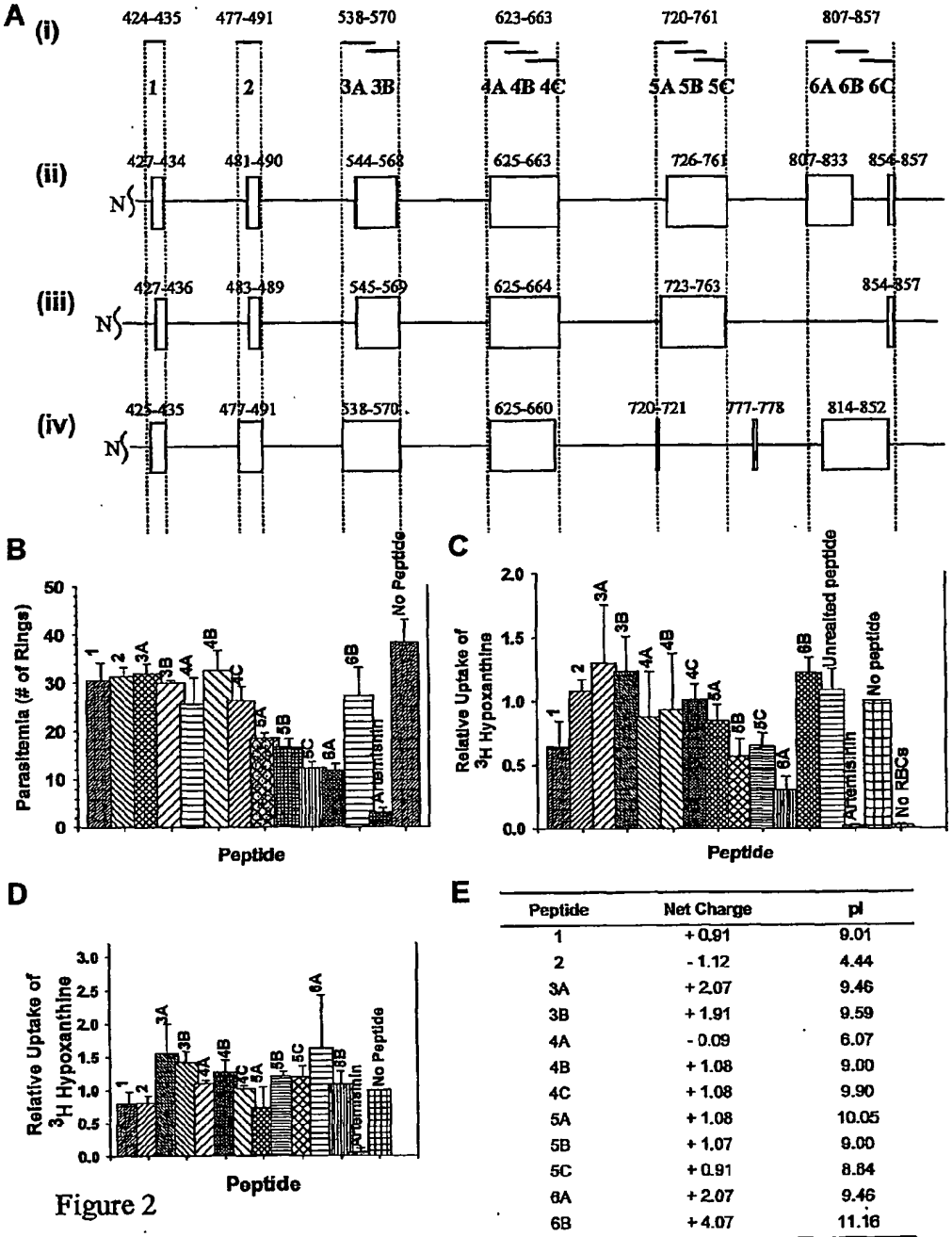

Figure 2

E

Peptide	Net Charge	pI
1	+ 0.91	9.01
2	- 1.12	4.44
3A	+ 2.07	9.46
3B	+ 1.91	9.59
4A	- 0.09	6.07
4B	+ 1.08	9.00
4C	+ 1.08	9.90
5A	+ 1.08	10.05
5B	+ 1.07	9.00
5C	+ 0.91	8.84
6A	+ 2.07	9.46
6B	+ 4.07	11.16

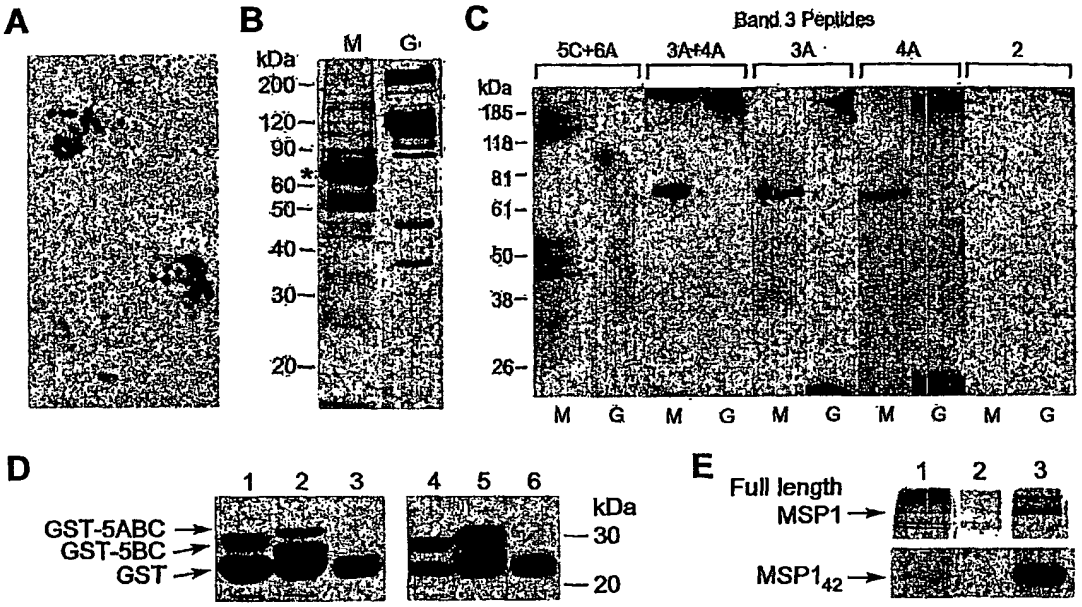
A
B
M
G
kDa
200
120
90
60
50
40
30
20
C
Band 3 Peptides
5C+6A
3A+4A
3A
4A
2
kDa
185
118
81
61
50
38
26
M G M G M G M G M G
D
1 2 3 4 5 6
kDa
GST-5ABC
GST-5BC
GST
30
20
E
Full length
MSP1
MSP1$_{42}$
1 2 3

Figure 3

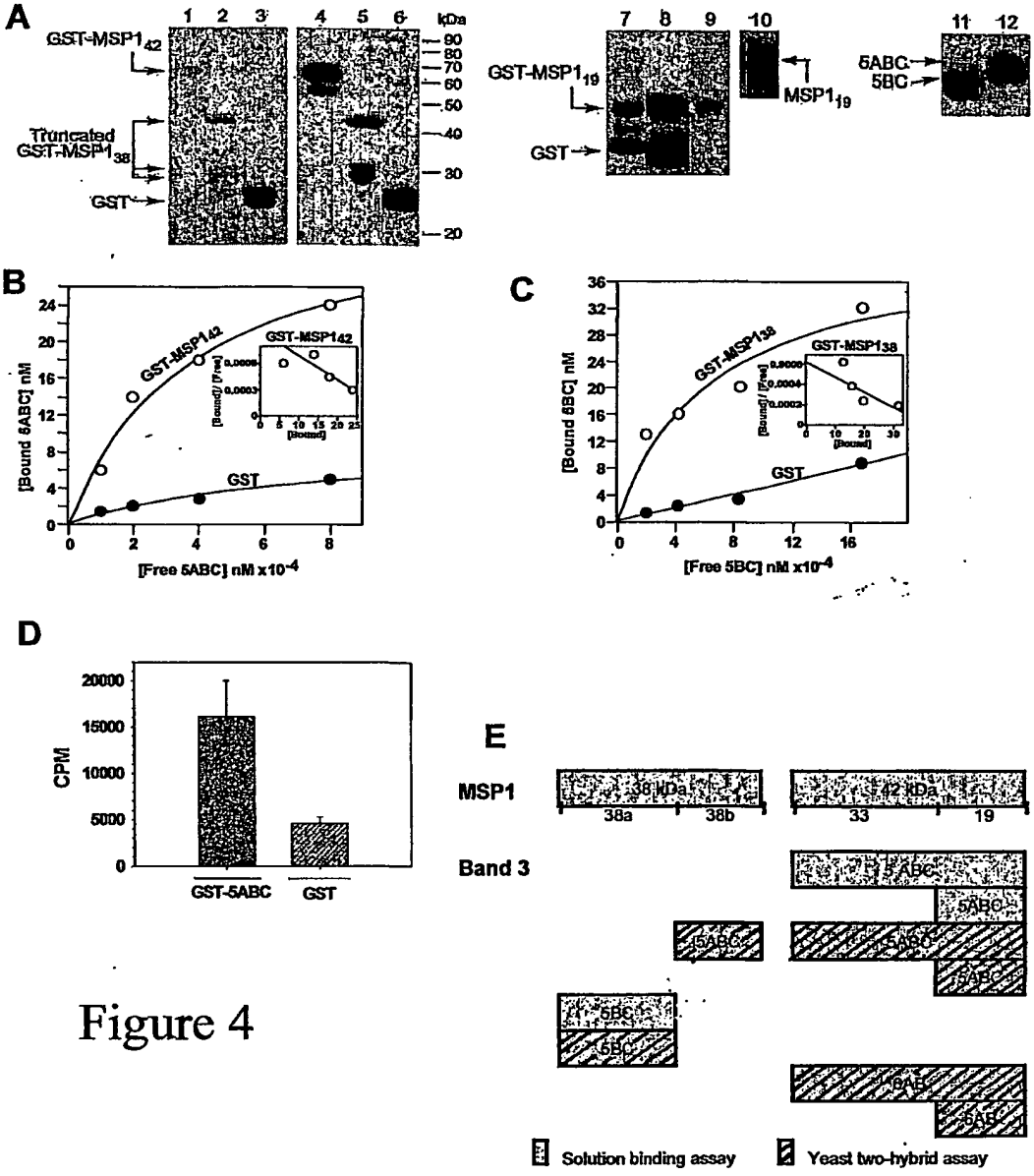
A
1 2 3 4 5 6 kDa
GST-MSP1 42
Truncated GST-MSP1 38
GST
90
80
70
60
50
40
30
20
7 8 9 10 11 12
GST-MSP1 19
MSP1 19
GST
5ABC
5BC
B
[Bound 5ABC] nM
GST-MSP1 42
GST
[Free 5ABC] nM x10-4
C
[Bound 5BC] nM
GST-MSP1 38
GST
[Free 5BC] nM x10-4
D
CPM
GST-5ABC
GST
E
MSP1
38 kDa
42 kDa
38a
38b
33
19
Band 3
5ABC
5BC
Solution binding assay
Yeast two-hybrid assay

Figure 4

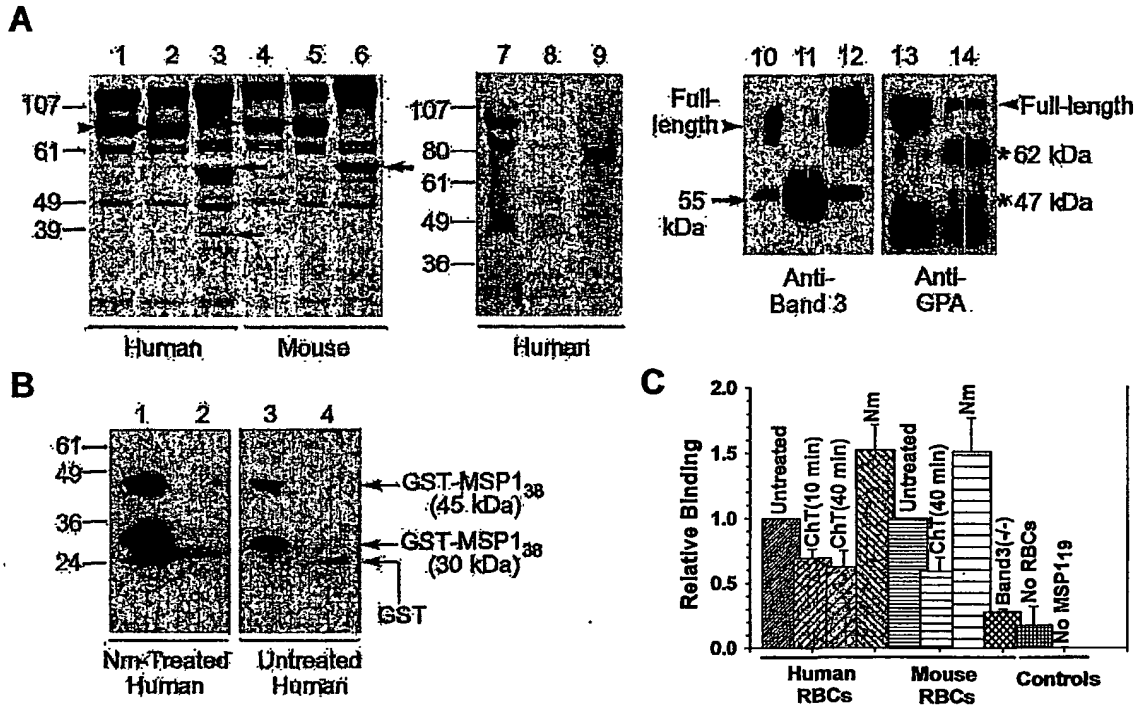
A
1 2 3 4 5 6
107
61
49
39
Human
Mouse
7 8 9
107
80
61
49
36
Human
10 11 12 13 14
Full-length
55 kDa
Anti-Band 3
Anti-GPA
Full-length
62 kDa
47 kDa
B
1 2 3 4
61
49
36
24
GST-MSP1 38 (45 kDa)
GST-MSP1 38 (30 kDa)
GST
Nm-Treated Human
Untreated Human
C
Relative Binding
2.0
1.5
1.0
0.5
0.0
Untreated
ChT(10 min)
ChT(40 min)
Nm
Untreated
ChT(40 min)
Nm
Band3(-/-)
No RBCs
No MSP119
Human RBCs
Mouse RBCs
Controls

Figure 5

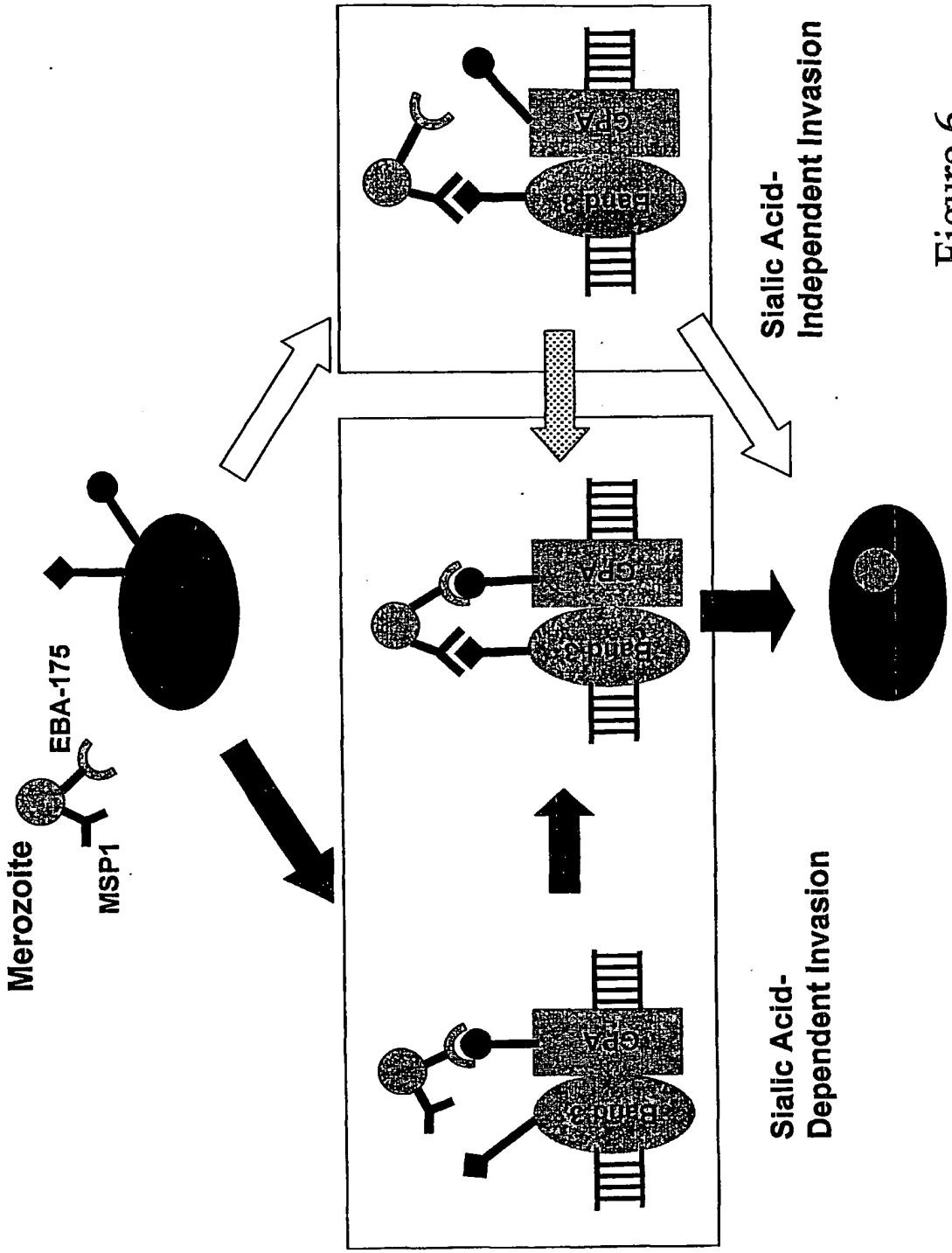
Merozoite
EBA-175
MSP1
GPA
Band 3
Sialic Acid-
Independent Invasion
Sialic Acid-
Dependent Invasion

Figure 6

SEQUENCE LISTING

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<130> S1237/7019WO

<150> US 06/272,930
<151> 2001-03-02

<160> 59

<170> PatentIn version 3.0

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Asn Ala Leu Thr
20

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Pro Gly Ala Ala
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<210> 3
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<213> Homo sapiens

<400> 3

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1 5 10 15

Glu Gln Arg Ile
20

<210> 4
<211> 20
<212> PRT
<213> Homo sapiens

<400> 4

Asp Arg Ile Leu Leu Leu Phe Lys Pro Pro Lys Tyr His Pro Asp Val
1 5 10 15

Pro Tyr Val Lys
20

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<211> 3475
<212> DNA
<213> Homo sapiens

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cgtagacaac tggacactca ggaccacgcc atggaggagc tgcaggatga ttatgaagac 180

atgatggagg agaatctgga gcaggaggaa tatgaagacc cagacatccc cgagtcccag 240

atggaggagc cggcagctca cgacaccgag gcaacagcca cagactacca caccacatca 300

cacccgggta cccacgaggt ctatgtggag ctgcaggagc tggtgatgga cgaaaagaac 360

caggagctga gatggatgga ggcggcgcgc tgggtgcaac tggaggagaa cctgggggag 420

aatggggcct ggggccgccc gcacctctct cacctcacct tctggagcct cctagagctg 480

cgtagagtct tcaccaaggg tactgtcctc ctagacctgc aagagacctc cctggctgga 540

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cactcaccat ctggaattct ggaaaagatt cccccggatt cagaggccac gttggtgcta 840

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gcactgctca gtctggtgcc tgtgcagagg gagctacttc gaaggcgcta tcagtccagc 1200

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gctctgctgg tcttcatcct catattcctg gagtctcaga tcaccacgct gattgtcagc 2220

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tggcgcatgc acttattcac gggcatccag atcatctgcc tggcagtgct gtgggtggtg 2700

aagtccacgc cggcctccct ggccctgccc ttcgtcctca tcctcactgt gccgctgcgg 2760

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Glu Pro Ala Ala His Asp Thr Glu Ala Thr Ala Thr Asp Tyr His Thr
35 40 45

Thr Ser His Pro Gly Thr His Glu Val Tyr Val Glu Leu Gln Glu Leu
50 55 60

Val Met Asp Glu Lys Asn Gln Glu Leu Arg Trp Met Glu Ala Ala Arg
65 70 75 80

Trp Val Gln Leu Glu Glu Asn Leu Gly Glu Asn Gly Ala Trp Gly Arg
85 90 95

Pro His Leu Ser His Leu Thr Phe Trp Ser Leu Leu Glu Leu Arg Arg
100 105 110

Val Phe Thr Lys Gly Thr Val Leu Leu Asp Leu Gln Glu Thr Ser Leu
115 120 125

Ala Gly Val Ala Asn Gln Leu Leu Asp Arg Phe Ile Phe Glu Asp Gln
130 135 140

Ile Arg Pro Gln Asp Arg Glu Glu Leu Leu Arg Ala Leu Leu Leu Lys
145 150 155 160

His Ser His Ala Gly Glu Leu Glu Ala Leu Gly Gly Val Lys Pro Ala
165 170 175

Val Leu Thr Arg Ser Gly Asp Pro Ser Gln Pro Leu Leu Pro Gln His
180 185 190

Ser Ser Leu Glu Thr Gln Leu Phe Cys Glu Gln Gly Asp Gly Gly Thr
195 200 205

Glu Gly His Ser Pro Ser Gly Ile Leu Glu Lys Ile Pro Pro Asp Ser
210 215 220

Glu Ala Thr Leu Val Leu Val Gly Arg Ala Asp Phe Leu Glu Gln Pro

225 230 235 240

Val Leu Gly Phe Val Arg Leu Gln Glu Ala Ala Glu Leu Glu Ala Val
245 250 255

Glu Leu Pro Val Pro Ile Arg Phe Leu Phe Val Leu Leu Gly Pro Glu
260 265 270

Ala Pro His Ile Asp Tyr Thr Gln Leu Gly Arg Ala Ala Ala Thr Leu
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Met Ser Glu Arg Val Phe Arg Ile Asp Ala Tyr Met Ala Gln Ser Arg
290 295 300

Gly Glu Leu Leu His Ser Leu Glu Gly Phe Leu Asp Cys Ser Leu Val
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Leu Pro Pro Thr Asp Ala Pro Ser Glu Gln Ala Leu Leu Ser Leu Val
325 330 335

Pro Val Gln Arg Glu Leu Leu Arg Arg Arg Tyr Gln Ser Ser Pro Ala
340 345 350

Lys Pro Asp Ser Ser Phe Tyr Lys Gly Leu Asp Leu Asn Gly Gly Pro
355 360 365

Asp Asp Pro Leu Gln Gln Thr Gly Gln Leu Phe Gly Gly Leu Val Arg
370 375 380

Asp Ile Arg Arg Arg Tyr Pro Tyr Tyr Leu Ser Asp Ile Thr Asp Ala
385 390 395 400

Phe Ser Pro Gln Val Leu Ala Ala Val Ile Phe Ile Tyr Phe Ala Ala
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Leu Ser Pro Ala Ile Thr Phe Gly Gly Leu Leu Gly Glu Lys Thr Arg
420 425 430

Asn Gln Met Gly Val Ser Glu Leu Leu Ile Ser Thr Ala Val Gln Gly
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Ile Leu Phe Ala Leu Leu Gly Ala Gln Pro Leu Leu Val Val Gly Phe
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Ser Gly Pro Leu Leu Val Phe Glu Glu Ala Phe Phe Ser Phe Cys Glu
465 470 475 480

Thr Asn Gly Leu Glu Tyr Ile Val Gly Arg Val Trp Ile Gly Phe Trp
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Leu Ile Leu Leu Val Val Leu Val Val Ala Phe Glu Gly Ser Phe Leu
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Val Arg Phe Ile Ser Arg Tyr Thr Gln Glu Ile Phe Ser Phe Leu Ile
515 520 525

Ser Leu Ile Phe Ile Tyr Glu Thr Phe Ser Lys Leu Ile Lys Ile Phe
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Gln Asp His Pro Leu Gln Lys Thr Tyr Asn Tyr Asn Val Leu Met Val

545 550 555 560

Pro Lys Pro Gln Gly Pro Leu Pro Asn Thr Ala Leu Leu Ser Leu Val
565 570 575

Leu Met Ala Gly Thr Phe Phe Phe Ala Met Met Leu Arg Lys Phe Lys
580 585 590

Asn Ser Ser Tyr Phe Pro Gly Lys Leu Arg Arg Val Ile Gly Asp Phe
595 600 605

Gly Val Pro Ile Ser Ile Leu Ile Met Val Leu Val Asp Phe Phe Ile
610 615 620

Gln Asp Thr Tyr Thr Gln Lys Leu Ser Val Pro Asp Gly Phe Lys Val
625 630 635 640

Ser Asn Ser Ser Ala Arg Gly Trp Val Ile His Pro Leu Gly Leu Arg
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Ser Glu Phe Pro Ile Trp Met Met Phe Ala Ser Ala Leu Pro Ala Leu
660 665 670

Leu Val Phe Ile Leu Ile Phe Leu Glu Ser Gln Ile Thr Thr Leu Ile
675 680 685

Val Ser Lys Pro Glu Arg Lys Met Val Lys Gly Ser Gly Phe His Leu
690 695 700

Asp Leu Leu Leu Val Val Gly Met Gly Gly Val Ala Ala Leu Phe Gly
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Met Pro Trp Leu Ser Ala Thr Thr Val Arg Ser Val Thr His Ala Asn
725 730 735

Ala Leu Thr Val Met Gly Lys Ala Ser Thr Pro Gly Ala Ala Ala Gln
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Ile Gln Glu Val Lys Glu Gln Arg Ile Ser Gly Leu Leu Val Ala Val
755 760 765

Leu Val Gly Leu Ser Ile Leu Met Glu Pro Ile Leu Ser Arg Ile Pro
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Leu Ala Val Leu Phe Gly Ile Phe Leu Tyr Met Gly Val Thr Ser Leu
785 790 795 800

Ser Gly Ile Gln Leu Phe Asp Arg Ile Leu Leu Leu Phe Lys Pro Pro
805 810 815

Lys Tyr His Pro Asp Val Pro Tyr Val Lys Arg Val Lys Thr Trp Arg
820 825 830

Met His Leu Phe Thr Gly Ile Gln Ile Ile Cys Leu Ala Val Leu Trp
835 840 845

Val Val Lys Ser Thr Pro Ala Ser Leu Ala Leu Pro Phe Val Leu Ile
850 855 860

Leu Thr Val Pro Leu Arg Arg Val Leu Leu Pro Leu Ile Phe Arg Asn

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Glu Pro Ala Ala His Asp Thr Glu Ala Thr Ala Thr Asp Tyr His Thr
35 40 45

Thr Ser His Pro Gly Thr His Lys Val Tyr Val Glu Leu Gln Glu Leu
50 55 60

Val Met Asp Glu Lys Asn Gln Glu Leu Arg Trp Met Glu Ala Ala Arg
65 70 75 80

Trp Val Gln Leu Glu Glu Asn Leu Gly Glu Asn Gly Ala Trp Gly Arg
85 90 95

Pro His Leu Ser His Leu Thr Phe Trp Ser Leu Leu Glu Leu Arg Arg
100 105 110

Val Phe Thr Lys Gly Thr Val Leu Leu Asp Leu Gln Glu Thr Ser Leu
115 120 125

Ala Gly Val Ala Asn Gln Leu Leu Asp Arg Phe Ile Phe Glu Asp Gln
130 135 140

Ile Arg Pro Gln Asp Arg Glu Glu Leu Leu Arg Ala Leu Leu Leu Lys
145 150 155 160

His Ser His Ala Gly Glu Leu Glu Ala Leu Gly Gly Val Lys Pro Ala
165 170 175

Val Leu Thr Arg Ser Gly Asp Pro Ser Gln Pro Leu Leu Pro Gln His
180 185 190

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Ser Ser Leu Glu Thr Gln Leu Phe Cys Glu Gln Gly Asp Gly Gly Thr
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Glu Gly His Ser Pro Ser Gly Ile Leu Glu Lys Ile Pro Pro Asp Ser
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Glu Ala Thr Leu Val Leu Val Gly Arg Ala Asp Phe Leu Glu Gln Pro
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Val Leu Gly Phe Val Arg Leu Gln Glu Ala Ala Glu Leu Glu Ala Val
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Glu Leu Pro Val Pro Ile Arg Phe Leu Phe Val Leu Leu Gly Pro Glu
            260                 265                 270

Ala Pro His Ile Asp Tyr Thr Gln Leu Gly Arg Ala Ala Ala Thr Leu
        275                 280                 285

Met Ser Glu Arg Val Phe Arg Ile Asp Ala Tyr Met Ala Gln Ser Arg
    290                 295                 300

Gly Glu Leu Leu His Ser Leu Glu Gly Phe Leu Asp Cys Ser Leu Val
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Leu Pro Pro Thr Asp Ala Pro Ser Glu Gln Ala Leu Leu Ser Leu Val
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Pro Val Gln Arg Glu Leu Leu Arg Arg Arg Tyr Gln Ser Ser Pro Ala
            340                 345                 350

Lys Pro Asp Ser Ser Phe Tyr Lys Gly Leu Asp Leu Asn Gly Gly Pro
        355                 360                 365

Asp Asp Pro Leu Gln Gln Thr Gly Gln Leu Phe Gly Gly Leu Val Arg
    370                 375                 380

Asp Ile Arg Arg Arg Tyr Pro Tyr Tyr Leu Ser Asp Ile Thr Asp Ala
385                 390                 395                 400

Phe Ser Pro Gln Val Leu Ala Ala Val Ile Phe Ile Tyr Phe Ala Ala
                405                 410                 415

Leu Ser Pro Ala Ile Thr Phe Gly Gly Leu Leu Gly Glu Lys Thr Arg
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Asn Gln Met Gly Val Ser Glu Leu Leu Ile Ser Thr Ala Val Gln Gly
        435                 440                 445

Ile Leu Phe Ala Leu Leu Gly Ala Gln Pro Leu Leu Val Val Gly Phe
    450                 455                 460

Ser Gly Pro Leu Leu Val Phe Glu Glu Ala Phe Phe Ser Phe Cys Glu
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Thr Asn Gly Leu Glu Tyr Ile Val Gly Arg Val Trp Ile Gly Phe Trp
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Leu Ile Leu Leu Val Val Leu Val Val Ala Phe Glu Gly Ser Phe Leu
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Ser Leu Ile Phe Ile Tyr Glu Thr Phe Ser Lys Leu Ile Lys Ile Phe
    530                 535                 540

Gln Asp His Pro Leu Gln Lys Thr Tyr Asn Tyr Asn Val Leu Met Val
545                 550                 555                 560

Pro Lys Pro Gln Gly Pro Leu Pro Asn Thr Ala Leu Leu Ser Leu Val
                565                 570                 575

Leu Met Ala Gly Thr Phe Phe Phe Ala Met Met Leu Arg Lys Phe Lys
            580                 585                 590

Asn Ser Ser Tyr Phe Pro Gly Lys Leu Arg Arg Val Ile Gly Asp Phe
        595                 600                 605

Gly Val Pro Ile Ser Ile Leu Ile Met Val Leu Val Asp Phe Phe Ile
    610                 615                 620

Gln Asp Thr Tyr Thr Gln Lys Leu Ser Val Pro Asp Gly Phe Lys Val
625                 630                 635                 640

Ser Asn Ser Ser Ala Arg Gly Trp Val Ile His Pro Leu Gly Leu Arg
                645                 650                 655

Ser Glu Phe Pro Ile Trp Met Met Phe Ala Ser Ala Leu Pro Ala Leu
            660                 665                 670

Leu Val Phe Ile Leu Ile Phe Leu Glu Ser Gln Ile Thr Thr Leu Ile
        675                 680                 685

Val Ser Lys Pro Glu Arg Lys Met Val Lys Gly Ser Gly Phe His Leu
    690                 695                 700

Asp Leu Leu Leu Val Val Gly Met Gly Gly Val Ala Ala Leu Phe Gly
705                 710                 715                 720

Met Pro Trp Leu Ser Ala Thr Thr Val Arg Ser Val Thr His Ala Asn
                725                 730                 735

Ala Leu Thr Val Met Gly Lys Ala Ser Thr Pro Gly Ala Ala Ala Gln
            740                 745                 750

Ile Gln Glu Val Lys Glu Gln Arg Ile Ser Gly Leu Leu Val Ala Val
        755                 760                 765

Leu Val Gly Leu Ser Ile Leu Met Glu Pro Ile Leu Ser Arg Ile Pro
    770                 775                 780

Leu Ala Val Leu Phe Gly Ile Phe Leu Tyr Met Gly Val Thr Ser Leu
785                 790                 795                 800

Ser Gly Ile Gln Leu Phe Asp Arg Ile Leu Leu Leu Phe Lys Pro Pro
                805                 810                 815

Lys Tyr His Pro Asp Val Pro Tyr Val Lys Arg Val Lys Thr Trp Arg
            820                 825                 830
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Met His Leu Phe Thr Gly Ile Gln Ile Ile Cys Leu Ala Val Leu Trp
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Val Val Lys Ser Thr Pro Ala Ser Leu Ala Leu Pro Phe Val Leu Ile
850 855 860

Leu Thr Val Pro Leu Arg Arg Val Leu Leu Pro Leu Ile Phe Arg Asn
865 870 875 880

Val Glu Leu Gln Cys Leu Asp Ala Asp Asp Ala Lys Ala Thr Phe Asp
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agtggatccg acacattaga acaaagtcaa ccaaagaaac cagcatcaac tcatgtagga 4080

gcagagtcta acacaataac aacatcacaa aatgtcgatg atgaagtaga tgacgtaatc 4140

atagtaccta tatttggaga atccgaagaa gattatgatg atttaggaca agtagtaaca 4200

ggagaagcag taactccttc cgtaattgat aacatacttt ctaaaattga aaatgaatat 4260

gaggttttat atttaaaacc tttagcaggt gtttatagaa gtttaaaaaa acaattagaa 4320

aataacgtta tgacatttaa tgttaatgtt aaggatattt taaattcacg atttaataaa 4380

cgtgaaaatt tcaaaaatgt tttagaatca gatttaattc catataaaga tttaacatca 4440

agtaattatg ttgtcaaaga tccatataaa tttcttaata aagaaaaaag agataaattc 4500

ttaagcagtt ataattatat taaggattca atagatacgg atataaattt tgcaaatgat 4560

gttcttggat attataaaat attatccgaa aaatataaat cagatttaga ttcaattaaa 4620

aaatatatca acgacaaaca aggtgaaaat gagaaatacc ttccctttt aaacaatatt 4680

gagaccttat ataaaacagt taatgataaa attgatttat ttgtaattca tttagaagca 4740

aaagttctaa attatacata tgagaaatca aacgtagaag ttaaaataaa agaacttaat 4800

tacttaaaaa caattcaaga caaattggca gattttaaaa aaaataacaa tttcgttgga 4860

attgctgatt tatcaacaga ttataaccat aataacttat tgacaaagtt ccttagtaca 4920

ggtatggttt ttgaaaatct tgctaaaacc gttttatcta atttacttga tggaaacttg 4980

caaggtatgt taaacatttc acaacaccaa tgcgtaaaaa aacaatgtcc acaaaattct 5040

ggatgtttca gacatttaga tgaaagagaa gaatgtaaat gtttattaaa ttacaaacaa 5100

gaaggtgata aatgtgttga aaatccaaat cctacttgta acgaaaataa tggtggatgt 5160

gatgcagatg ccaaatgtac cgaagaagat tcaggtagca acggaaagaa aatcacatgt 5220

gaatgtacta aacctgattc ttatccactt ttcgatggta ttttctgcag ttcctctaac 5280

ttcttaggaa tatcattctt attaatactc atgttaatat tatacagttt catttaaaaa 5340

atgtaggagt taaaatatgt taccttaatt ttttttttt ttttttttt taaatatata 5400

tatatattaa tatatatata taaaatatta cataatatat atatatatat ttagttatta 5460

caggaatagt gatattttag tcatgttcaa aatatattaa aaaattataa atattataat 5520

aaaaaaaaaa aaaaaaaaaa attatatact tataaattta tacatttata catatatata 5580

tatatatttt tttttcttct ttcttttcaa gttctatttt atattttata tatagattta 5640

ataaaaaact ttttaaaata aaaaaaagta cgtaaatttt aatatatata tatatatata 5700

taatatatat ataatatata tttatttatt tatatgtata atatatttac atatattatt 5760

ataatatata tatttttttt tacgcataca taaaaagcat ttttttttt tataaacatt 5820

ccaacaatta taaaataact ttaataataa cattaaattt ttatttttt ttttaaaaaa 5880

aaaaaaaaaa aaaaaaaaaa actaaagaga ttattca 5917

<210> 10
<211> 1639
<212> PRT
<213> Plasmodium falciparum

<400> 10

Met Lys Ile Ile Phe Phe Leu Cys Ser Phe Leu Phe Phe Ile Ile Asn
1 5 10 15

Thr Gln Cys Val Thr His Glu Ser Tyr Gln Glu Leu Val Lys Lys Leu
20 25 30

Glu Ala Leu Glu Asp Ala Val Leu Thr Gly Tyr Ser Leu Phe Gln Lys
35 40 45

Glu Lys Met Val Leu Asn Glu Gly Thr Ser Gly Thr Ala Val Thr Thr
50 55 60

Ser Thr Pro Gly Ser Lys Gly Ser Val Ala Ser Gly Gly Ser Gly Gly

65 70 75 80

Ser Val Ala Ser Gly Gly Ser Val Ala Ser Gly Gly Ser Val Ala Ser
85 90 95

Gly Gly Ser Val Ala Ser Gly Gly Ser Gly Asn Ser Arg Arg Thr Asn
100 105 110

Pro Ser Asp Asn Ser Ser Asp Ser Asp Ala Lys Ser Tyr Ala Asp Leu
115 120 125

Lys His Arg Val Arg Asn Tyr Leu Leu Thr Ile Lys Glu Leu Lys Tyr
130 135 140

Pro Gln Leu Phe Asp Leu Thr Asn His Met Leu Thr Leu Cys Asp Asn
145 150 155 160

Ile His Gly Phe Lys Tyr Leu Ile Asp Gly Tyr Glu Glu Ile Asn Glu
165 170 175

Leu Leu Tyr Lys Leu Asn Phe Tyr Phe Asp Leu Leu Arg Ala Lys Leu
180 185 190

Asn Asp Val Cys Ala Asn Asp Tyr Cys Gln Ile Pro Phe Asn Leu Lys
195 200 205

Ile Arg Ala Asn Glu Leu Asp Val Leu Lys Lys Leu Val Phe Gly Tyr
210 215 220

Arg Lys Pro Leu Asp Asn Ile Lys Asp Asn Val Gly Lys Met Glu Asp
225 230 235 240

Tyr Ile Lys Lys Asn Lys Lys Thr Ile Glu Asn Ile Asn Glu Leu Ile
245 250 255

Glu Glu Ser Lys Lys Thr Ile Asp Lys Asn Lys Asn Ala Thr Lys Glu
260 265 270

Glu Glu Lys Lys Lys Leu Tyr Gln Ala Gln Tyr Asp Leu Ser Ile Tyr
275 280 285

Asn Lys Gln Leu Glu Glu Ala His Asn Leu Ile Ser Val Leu Glu Lys
290 295 300

Arg Ile Asp Thr Leu Lys Lys Asn Glu Asn Ile Lys Glu Leu Leu Asp
305 310 315 320

Lys Ile Asn Glu Ile Lys Asn Pro Pro Pro Ala Asn Ser Gly Asn Thr
325 330 335

Pro Asn Thr Leu Leu Asp Lys Asn Lys Lys Ile Glu Glu His Glu Lys
340 345 350

Glu Ile Lys Glu Ile Ala Lys Thr Ile Lys Phe Asn Ile Asp Ser Leu
355 360 365

Phe Thr Asp Pro Leu Glu Leu Glu Tyr Tyr Leu Arg Glu Lys Asn Lys
370 375 380

Asn Ile Asp Ile Ser Ala Lys Val Glu Thr Lys Glu Ser Thr Glu Pro

385 390 395 400

Asn Glu Tyr Pro Asn Gly Val Thr Tyr Pro Leu Ser Tyr Asn Asp Ile
405 410 415

Asn Asn Ala Leu Asn Glu Leu Asn Ser Phe Gly Asp Leu Ile Asn Pro
420 425 430

Phe Asp Tyr Thr Lys Glu Pro Ser Lys Asn Ile Tyr Thr Asp Asn Glu
435 440 445

Arg Lys Lys Phe Ile Asn Glu Ile Lys Glu Lys Ile Lys Ile Glu Lys
450 455 460

Lys Lys Ile Glu Ser Asp Lys Lys Ser Tyr Glu Asp Arg Ser Lys Ser
465 470 475 480

Leu Asn Asp Ile Thr Lys Glu Tyr Glu Lys Leu Leu Asn Glu Ile Tyr
485 490 495

Asp Ser Lys Phe Asn Asn Asn Ile Asp Leu Thr Asn Phe Glu Lys Met
500 505 510

Met Gly Lys Arg Tyr Ser Tyr Lys Val Glu Lys Leu Thr His His Asn
515 520 525

Thr Phe Ala Ser Tyr Glu Asn Ser Lys His Asn Leu Glu Lys Leu Thr
530 535 540

Lys Ala Leu Lys Tyr Met Glu Asp Tyr Ser Leu Arg Asn Ile Val Val
545 550 555 560

Glu Lys Glu Leu Lys Tyr Tyr Lys Asn Leu Ile Ser Lys Ile Glu Asn
565 570 575

Glu Ile Glu Thr Leu Val Glu Asn Ile Lys Lys Asp Glu Glu Gln Leu
580 585 590

Phe Glu Lys Lys Ile Thr Lys Asp Glu Asn Lys Pro Asp Glu Lys Ile
595 600 605

Leu Glu Val Ser Asp Ile Val Lys Val Gln Val Gln Lys Val Leu Leu
610 615 620

Met Asn Lys Ile Asp Glu Leu Lys Lys Thr Gln Leu Ile Leu Lys Asn
625 630 635 640

Val Glu Leu Lys His Asn Ile His Val Pro Asn Ser Tyr Lys Gln Glu
645 650 655

Asn Lys Gln Glu Pro Tyr Tyr Leu Ile Val Leu Lys Lys Glu Ile Asp
660 665 670

Lys Leu Lys Val Phe Met Pro Lys Val Glu Ser Leu Ile Asn Glu Glu
675 680 685

Lys Lys Asn Ile Lys Thr Glu Gly Gln Ser Asp Asn Ser Glu Pro Ser
690 695 700

Thr Glu Gly Glu Ile Thr Gly Gln Ala Thr Thr Lys Pro Gly Gln Gln

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705                 710                 715                 720

Ala Gly Ser Ala Leu Glu Gly Asp Ser Val Gln Ala Gln Ala Gln Glu
                725                 730                 735

Gln Lys Gln Ala Gln Pro Pro Val Pro Val Pro Val Pro Glu Ala Lys
            740                 745                 750

Ala Gln Val Pro Thr Pro Pro Ala Pro Val Asn Asn Lys Thr Glu Asn
        755                 760                 765

Val Ser Lys Leu Asp Tyr Leu Glu Lys Leu Tyr Glu Phe Leu Asn Thr
    770                 775                 780

Ser Tyr Ile Cys His Lys Tyr Ile Leu Val Ser His Ser Thr Met Asn
785                 790                 795                 800

Glu Lys Ile Leu Lys Gln Tyr Lys Ile Thr Lys Glu Glu Glu Ser Lys
                805                 810                 815

Leu Ser Ser Cys Asp Pro Leu Asp Leu Leu Phe Asn Ile Gln Asn Asn
            820                 825                 830

Ile Pro Val Met Tyr Ser Met Phe Asp Ser Leu Asn Asn Ser Leu Ser
        835                 840                 845

Gln Leu Phe Met Glu Ile Tyr Glu Lys Glu Met Val Cys Asn Leu Tyr
    850                 855                 860

Lys Leu Lys Asp Asn Asp Lys Ile Lys Asn Leu Leu Glu Glu Ala Lys
865                 870                 875                 880

Lys Val Ser Thr Ser Val Lys Thr Leu Ser Ser Ser Ser Met Gln Pro
                885                 890                 895

Leu Ser Leu Thr Pro Gln Asp Lys Pro Glu Val Ser Ala Asn Asp Asp
            900                 905                 910

Thr Ser His Ser Thr Asn Leu Asn Asn Ser Leu Lys Leu Phe Glu Asn
        915                 920                 925

Ile Leu Ser Leu Gly Lys Asn Lys Asn Ile Tyr Gln Glu Leu Ile Gly
    930                 935                 940

Gln Lys Ser Ser Glu Asn Phe Tyr Glu Lys Ile Leu Lys Asp Ser Asp
945                 950                 955                 960

Thr Phe Tyr Asn Glu Ser Phe Thr Asn Phe Val Lys Ser Lys Ala Asp
                965                 970                 975

Asp Ile Asn Ser Leu Asn Asp Glu Ser Lys Arg Lys Lys Leu Glu Glu
            980                 985                 990

Asp Ile Asn Lys Leu Lys Lys Thr  Leu Gln Leu Ser Phe  Asp Leu Tyr
        995                 1000                1005

Asn Lys  Tyr Lys Leu Lys Leu  Glu Arg Leu Phe Asp  Lys Lys Lys
    1010                1015                1020

Thr Val  Gly Lys Tyr Lys Met  Gln Ile Lys Lys Leu  Thr Leu Leu
```

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    1025                1030                1035

Lys Glu  Gln Leu Glu Ser Lys  Leu Asn Ser Leu Asn  Asn Pro Lys
    1040                1045                1050

His Val  Leu Gln Asn Phe Ser  Val Phe Phe Asn Lys  Lys Lys Glu
    1055                1060                1065

Ala Glu  Ile Ala Glu Thr Glu  Asn Thr Leu Glu Asn  Thr Lys Ile
    1070                1075                1080

Leu Leu  Lys His Tyr Lys Gly  Leu Val Lys Tyr Tyr  Asn Gly Glu
    1085                1090                1095

Ser Ser  Pro Leu Lys Thr Leu  Ser Glu Glu Ser Ile  Gln Thr Glu
    1100                1105                1110

Asp Asn  Tyr Ala Ser Leu Glu  Asn Phe Lys Val Leu  Ser Lys Leu
    1115                1120                1125

Glu Gly  Lys Leu Lys Asp Asn  Leu Asn Leu Glu Lys  Lys Lys Leu
    1130                1135                1140

Ser Tyr  Leu Ser Ser Gly Leu  His His Leu Ile Ala  Glu Leu Lys
    1145                1150                1155

Glu Val  Ile Lys Asn Lys Asn  Tyr Thr Gly Asn Ser  Pro Ser Glu
    1160                1165                1170

Asn Asn  Thr Asp Val Asn Asn  Ala Leu Glu Ser Tyr  Lys Lys Phe
    1175                1180                1185

Leu Pro  Glu Gly Thr Asp Val  Ala Thr Val Val Ser  Glu Ser Gly
    1190                1195                1200

Ser Asp  Thr Leu Glu Gln Ser  Gln Pro Lys Lys Pro  Ala Ser Thr
    1205                1210                1215

His Val  Gly Ala Glu Ser Asn  Thr Ile Thr Thr Ser  Gln Asn Val
    1220                1225                1230

Asp Asp  Glu Val Asp Asp Val  Ile Ile Val Pro Ile  Phe Gly Glu
    1235                1240                1245

Ser Glu  Glu Asp Tyr Asp Asp  Leu Gly Gln Val Val  Thr Gly Glu
    1250                1255                1260

Ala Val  Thr Pro Ser Val Ile  Asp Asn Ile Leu Ser  Lys Ile Glu
    1265                1270                1275

Asn Glu  Tyr Glu Val Leu Tyr  Leu Lys Pro Leu Ala  Gly Val Tyr
    1280                1285                1290

Arg Ser  Leu Lys Lys Gln Leu  Glu Asn Asn Val Met  Thr Phe Asn
    1295                1300                1305

Val Asn  Val Lys Asp Ile Leu  Asn Ser Arg Phe Asn  Lys Arg Glu
    1310                1315                1320

Asn Phe  Lys Asn Val Leu Glu  Ser Asp Leu Ile Pro  Tyr Lys Asp
```

1325 1330 1335

Leu Thr Ser Ser Asn Tyr Val Val Lys Asp Pro Tyr Lys Phe Leu
1340 1345 1350

Asn Lys Glu Lys Arg Asp Lys Phe Leu Ser Ser Tyr Asn Tyr Ile
1355 1360 1365

Lys Asp Ser Ile Asp Thr Asp Ile Asn Phe Ala Asn Asp Val Leu
1370 1375 1380

Gly Tyr Tyr Lys Ile Leu Ser Glu Lys Tyr Lys Ser Asp Leu Asp
1385 1390 1395

Ser Ile Lys Lys Tyr Ile Asn Asp Lys Gln Gly Glu Asn Glu Lys
1400 1405 1410

Tyr Leu Pro Phe Leu Asn Asn Ile Glu Thr Leu Tyr Lys Thr Val
1415 1420 1425

Asn Asp Lys Ile Asp Leu Phe Val Ile His Leu Glu Ala Lys Val
1430 1435 1440

Leu Asn Tyr Thr Tyr Glu Lys Ser Asn Val Glu Val Lys Ile Lys
1445 1450 1455

Glu Leu Asn Tyr Leu Lys Thr Ile Gln Asp Lys Leu Ala Asp Phe
1460 1465 1470

Lys Lys Asn Asn Asn Phe Val Gly Ile Ala Asp Leu Ser Thr Asp
1475 1480 1485

Tyr Asn His Asn Asn Leu Leu Thr Lys Phe Leu Ser Thr Gly Met
1490 1495 1500

Val Phe Glu Asn Leu Ala Lys Thr Val Leu Ser Asn Leu Leu Asp
1505 1510 1515

Gly Asn Leu Gln Gly Met Leu Asn Ile Ser Gln His Gln Cys Val
1520 1525 1530

Lys Lys Gln Cys Pro Gln Asn Ser Gly Cys Phe Arg His Leu Asp
1535 1540 1545

Glu Arg Glu Glu Cys Lys Cys Leu Leu Asn Tyr Lys Gln Glu Gly
1550 1555 1560

Asp Lys Cys Val Glu Asn Pro Asn Pro Thr Cys Asn Glu Asn Asn
1565 1570 1575

Gly Gly Cys Asp Ala Asp Ala Lys Cys Thr Glu Glu Asp Ser Gly
1580 1585 1590

Ser Asn Gly Lys Lys Ile Thr Cys Glu Cys Thr Lys Pro Asp Ser
1595 1600 1605

Tyr Pro Leu Phe Asp Gly Ile Phe Cys Ser Ser Ser Asn Phe Leu
1610 1615 1620

Gly Ile Ser Phe Leu Leu Ile Leu Met Leu Ile Leu Tyr Ser Phe

1625 1630 1635

Ile

<210> 11
<211> 378
<212> PRT
<213> Plasmodium falciparum

<400> 11

Gly Glu Ala Val Thr Pro Ser Val Ile Asp Asn Ile Leu Ser Lys Ile
1 5 10 15

Glu Asn Glu Tyr Glu Val Leu Tyr Leu Lys Pro Leu Ala Gly Val Tyr
20 25 30

Arg Ser Leu Lys Lys Gln Leu Glu Asn Asn Val Met Thr Phe Asn Val
35 40 45

Asn Val Lys Asp Ile Leu Asn Ser Arg Phe Asn Lys Arg Glu Asn Phe
50 55 60

Lys Asn Val Leu Glu Ser Asp Leu Ile Pro Tyr Lys Asp Leu Thr Ser
65 70 75 80

Ser Asn Tyr Val Val Lys Asp Pro Tyr Lys Phe Leu Asn Lys Glu Lys
85 90 95

Arg Asp Lys Phe Leu Ser Ser Tyr Asn Tyr Ile Lys Asp Ser Ile Asp
100 105 110

Thr Asp Ile Asn Phe Ala Asn Asp Val Leu Gly Tyr Tyr Lys Ile Leu
115 120 125

Ser Glu Lys Tyr Lys Ser Asp Leu Asp Ser Ile Lys Lys Tyr Ile Asn
130 135 140

Asp Lys Gln Gly Glu Asn Glu Lys Tyr Leu Pro Phe Leu Asn Asn Ile
145 150 155 160

Glu Thr Leu Tyr Lys Thr Val Asn Asp Lys Ile Asp Leu Phe Val Ile
165 170 175

His Leu Glu Ala Lys Val Leu Asn Tyr Thr Tyr Glu Lys Ser Asn Val
180 185 190

Glu Val Lys Ile Lys Glu Leu Asn Tyr Leu Lys Thr Ile Gln Asp Lys
195 200 205

Leu Ala Asp Phe Lys Lys Asn Asn Asn Phe Val Gly Ile Ala Asp Leu
210 215 220

Ser Thr Asp Tyr Asn His Asn Asn Leu Leu Thr Lys Phe Leu Ser Thr
225 230 235 240

Gly Met Val Phe Glu Asn Leu Ala Lys Thr Val Leu Ser Asn Leu Leu
245 250 255

```
Asp Gly Asn Leu Gln Gly Met Leu Asn Ile Ser Gln His Gln Cys Val
            260                 265                 270

Lys Lys Gln Cys Pro Gln Asn Ser Gly Cys Phe Arg His Leu Asp Glu
        275                 280                 285

Arg Glu Glu Cys Lys Cys Leu Leu Asn Tyr Lys Gln Glu Gly Asp Lys
    290                 295                 300

Cys Val Glu Asn Pro Asn Pro Thr Cys Asn Glu Asn Asn Gly Gly Cys
305                 310                 315                 320

Asp Ala Asp Ala Lys Cys Thr Glu Glu Asp Ser Gly Ser Asn Gly Lys
                325                 330                 335

Lys Ile Thr Cys Glu Cys Thr Lys Pro Asp Ser Tyr Pro Leu Phe Asp
            340                 345                 350

Gly Ile Phe Cys Ser Ser Ser Asn Phe Leu Gly Ile Ser Phe Leu Leu
        355                 360                 365

Ile Leu Met Leu Ile Leu Tyr Ser Phe Ile
    370                 375
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<210> 12
<211> 360
<212> PRT
<213> Plasmodium falciparum

<400> 12

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Gln Asp Lys Pro Glu Val Ser Ala Asn Asp Asp Thr Ser His Ser Thr
1               5                   10                  15

Asn Leu Asn Asn Ser Leu Lys Leu Phe Glu Asn Ile Leu Ser Leu Gly
            20                  25                  30

Lys Asn Lys Asn Ile Tyr Gln Glu Leu Ile Gly Gln Lys Ser Ser Glu
        35                  40                  45

Asn Phe Tyr Glu Lys Ile Leu Lys Asp Ser Asp Thr Phe Tyr Asn Glu
    50                  55                  60

Ser Phe Thr Asn Phe Val Lys Ser Lys Ala Asp Asp Ile Asn Ser Leu
65                  70                  75                  80

Asn Asp Glu Ser Lys Arg Lys Lys Leu Glu Glu Asp Ile Asn Lys Leu
                85                  90                  95

Lys Lys Thr Leu Gln Leu Ser Phe Asp Leu Tyr Asn Lys Tyr Lys Leu
            100                 105                 110

Lys Leu Glu Arg Leu Phe Asp Lys Lys Lys Thr Val Gly Lys Tyr Lys
        115                 120                 125

Met Gln Ile Lys Lys Leu Thr Leu Leu Lys Glu Gln Leu Glu Ser Lys
    130                 135                 140

Leu Asn Ser Leu Asn Asn Pro Lys His Val Leu Gln Asn Phe Ser Val
145                 150                 155                 160
```

```
Phe Phe Asn Lys Lys Lys Glu Ala Glu Ile Ala Glu Thr Glu Asn Thr
                165                 170                 175

Leu Glu Asn Thr Lys Ile Leu Leu Lys His Tyr Lys Gly Leu Val Lys
            180                 185                 190

Tyr Tyr Asn Gly Glu Ser Ser Pro Leu Lys Thr Leu Ser Glu Glu Ser
        195                 200                 205

Ile Gln Thr Glu Asp Asn Tyr Ala Ser Leu Glu Asn Phe Lys Val Leu
    210                 215                 220

Ser Lys Leu Glu Gly Lys Leu Lys Asp Asn Leu Asn Leu Glu Lys Lys
225                 230                 235                 240

Lys Leu Ser Tyr Leu Ser Ser Gly Leu His His Leu Ile Ala Glu Leu
                245                 250                 255

Lys Glu Val Ile Lys Asn Lys Asn Tyr Thr Gly Asn Ser Pro Ser Glu
            260                 265                 270

Asn Asn Thr Asp Val Asn Asn Ala Leu Glu Ser Tyr Lys Lys Phe Leu
        275                 280                 285

Pro Glu Gly Thr Asp Val Ala Thr Val Val Ser Glu Ser Gly Ser Asp
    290                 295                 300

Thr Leu Glu Gln Ser Gln Pro Lys Lys Pro Ala Ser Thr His Val Gly
305                 310                 315                 320

Ala Glu Ser Asn Thr Ile Thr Thr Ser Gln Asn Val Asp Asp Glu Val
                325                 330                 335

Asp Asp Val Ile Ile Val Pro Ile Phe Gly Glu Ser Glu Glu Asp Tyr
            340                 345                 350

Asp Asp Leu Gly Gln Val Val Thr
        355                 360
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<210> 13
<211> 220
<212> PRT
<213> Plasmodium falciparum

<400> 13

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Gln Asp Lys Pro Glu Val Ser Ala Asn Asp Asp Thr Ser His Ser Thr
1               5                   10                  15

Asn Leu Asn Asn Ser Leu Lys Leu Phe Glu Asn Ile Leu Ser Leu Gly
            20                  25                  30

Lys Asn Lys Asn Ile Tyr Gln Glu Leu Ile Gly Gln Lys Ser Ser Glu
        35                  40                  45

Asn Phe Tyr Glu Lys Ile Leu Lys Asp Ser Asp Thr Phe Tyr Asn Glu
    50                  55                  60

Ser Phe Thr Asn Phe Val Lys Ser Lys Ala Asp Asp Ile Asn Ser Leu
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65 70 75 80

Asn Asp Glu Ser Lys Arg Lys Lys Leu Glu Glu Asp Ile Asn Lys Leu
85 90 95

Lys Lys Thr Leu Gln Leu Ser Phe Asp Leu Tyr Asn Lys Tyr Lys Leu
100 105 110

Lys Leu Glu Arg Leu Phe Asp Lys Lys Lys Thr Val Gly Lys Tyr Lys
115 120 125

Met Gln Ile Lys Lys Leu Thr Leu Leu Lys Glu Gln Leu Glu Ser Lys
130 135 140

Leu Asn Ser Leu Asn Asn Pro Lys His Val Leu Gln Asn Phe Ser Val
145 150 155 160

Phe Phe Asn Lys Lys Lys Glu Ala Glu Ile Ala Glu Thr Glu Asn Thr
165 170 175

Leu Glu Asn Thr Lys Ile Leu Leu Lys His Tyr Lys Gly Leu Val Lys
180 185 190

Tyr Tyr Asn Gly Glu Ser Ser Pro Leu Lys Thr Leu Ser Glu Glu Ser
195 200 205

Ile Gln Thr Glu Asp Asn Tyr Ala Ser Leu Glu Asn
210 215 220

<210> 14
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 14
ctcgagctca ggataaaccc 20

<210> 15
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 15
gcggccgcac ttgttagt 18

<210> 16
<211> 23
<212> DNA

<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 16
ctcgagctgg agaagcagta act 23

<210> 17
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 17
gcggccgcac taaatgaaac tgtata 26

<210> 18
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 18
ccgggatcca acatttcaca acaccaa 27

<210> 19
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 19
ccggaattca atgaaactgt ataata 26

<210> 20
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature

<223> Synthetic Oligonucleotide

<400> 20
ccgggatccg ggatgccctg gctcagtgcc a 31

<210> 21
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 21
ccggaattct tagatccgct gctctttgac ctc 33

<210> 22
<211> 42
<212> PRT
<213> Homo sapien

<400> 22

Gly Met Pro Trp Leu Ser Ala Thr Thr Val Arg Ser Val Thr His Ala
1 5 10 15

Asn Ala Leu Thr Val Met Gly Lys Ala Ser Thr Pro Gly Ala Ala Ala
20 25 30

Gln Ile Gln Glu Val Lys Glu Gln Arg Ile
35 40

<210> 23
<211> 51
<212> PRT
<213> Homo sapien

<400> 23

Asp Arg Ile Leu Leu Leu Phe Lys Pro Pro Lys Tyr His Pro Asp Val
1 5 10 15

Pro Tyr Val Lys Arg Val Lys Thr Trp Arg Met His Leu Phe Thr Gly
20 25 30

Ile Gln Ile Ile Cys Leu Ala Val Leu Trp Val Val Lys Ser Thr Pro
35 40 45

Ala Ser Leu
50

<210> 24
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 24
ccgggatcct ccgtcaccca tgccaacgcc 30

<210> 25
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 25
ccgggatccg accgcatctt gcttctgttc a 31

<210> 26
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 26
ccggaattct tagatctgga tgcccgtgaa 30

<210> 27
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 27
ggccatatgg atgatacatc acatt 25

<210> 28
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 28
ggcctcgagg ttttctaaac tggcat 26

<210> 29
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 29
ggccatatgt ttaaagtatt aagta 25

<210> 30
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
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<400> 30
ggcctcgagt tctcctgtta ctacttg 27

<210> 31
<211> 25
<212> DNA
<213> Artificial Sequence

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<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 31
gccgaattcg cagtaactcc ttccg 25

<210> 32
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 32
gccggatcca atgaaactgt ataata 26

<210> 33
<211> 334
<212> PRT
<213> Plasmodium falciparum

<400> 33

Gln Asp Lys Pro Glu Val Ser Ala Asn Asp Asp Thr Ser His Ser Thr
1 5 10 15

Asn Leu Asn Asn Ser Leu Lys Leu Phe Glu Asn Ile Leu Ser Leu Gly
20 25 30

Lys Asn Lys Asn Ile Tyr Gln Glu Leu Ile Gly Gln Lys Ser Ser Glu
35 40 45

Asn Phe Tyr Glu Lys Ile Leu Lys Asp Ser Asp Thr Phe Tyr Asn Glu
50 55 60

Ser Phe Thr Asn Phe Val Lys Ser Lys Ala Asp Asp Ile Asn Ser Leu
65 70 75 80

Asn Asp Glu Ser Lys Arg Lys Lys Leu Glu Glu Asp Ile Asn Lys Leu
85 90 95

Lys Lys Thr Leu Gln Leu Ser Phe Asp Leu Tyr Asn Lys Tyr Lys Leu
100 105 110

Lys Leu Glu Arg Leu Phe Asp Lys Lys Lys Thr Val Gly Lys Tyr Lys
115 120 125

Met Gln Ile Lys Lys Leu Thr Leu Leu Lys Glu Gln Leu Glu Ser Lys
130 135 140

Leu Asn Ser Leu Asn Asn Pro Lys His Val Leu Gln Asn Phe Ser Val
145 150 155 160

Phe Phe Asn Lys Lys Lys Glu Ala Glu Ile Ala Glu Thr Glu Asn Thr
165 170 175

Leu Glu Asn Thr Lys Ile Leu Leu Lys His Tyr Lys Gly Leu Val Lys
180 185 190

Tyr Tyr Asn Gly Glu Ser Ser Pro Leu Lys Thr Leu Ser Glu Glu Ser
195 200 205

Ile Gln Thr Glu Asp Asn Tyr Ala Ser Leu Glu Asn Phe Lys Val Leu
210 215 220

Ser Lys Leu Glu Gly Lys Leu Lys Asp Asn Leu Asn Leu Glu Lys Lys
225 230 235 240

Lys Leu Ser Tyr Leu Ser Ser Gly Leu His His Leu Ile Ala Glu Leu
245 250 255

Lys Glu Val Ile Lys Asn Lys Asn Tyr Thr Gly Asn Ser Pro Ser Glu
260 265 270

Asn Asn Thr Asp Val Asn Asn Ala Leu Glu Ser Tyr Lys Lys Phe Leu
275 280 285

Pro Glu Gly Thr Asp Val Ala Thr Val Val Ser Glu Ser Gly Ser Asp
290 295 300

Thr Leu Glu Gln Ser Gln Pro Lys Lys Pro Ala Ser Thr His Val Gly
305 310 315 320

Ala Glu Ser Asn Thr Ile Thr Thr Ser Gln Asn Val Asp Asp
325 330

<210> 34
<211> 376
<212> PRT
<213> Plasmodium falciparum

<400> 34

Ala Val Thr Pro Ser Val Ile Asp Asn Ile Leu Ser Lys Ile Glu Asn
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Glu Tyr Glu Val Leu Tyr Leu Lys Pro Leu Ala Gly Val Tyr Arg Ser
20 25 30

Leu Lys Lys Gln Leu Glu Asn Asn Val Met Thr Phe Asn Val Asn Val
35 40 45

Lys Asp Ile Leu Asn Ser Arg Phe Asn Lys Arg Glu Asn Phe Lys Asn
50 55 60

Val Leu Glu Ser Asp Leu Ile Pro Tyr Lys Asp Leu Thr Ser Ser Asn
65 70 75 80

Tyr Val Val Lys Asp Pro Tyr Lys Phe Leu Asn Lys Glu Lys Arg Asp
85 90 95

Lys Phe Leu Ser Ser Tyr Asn Tyr Ile Lys Asp Ser Ile Asp Thr Asp
100 105 110

Ile Asn Phe Ala Asn Asp Val Leu Gly Tyr Tyr Lys Ile Leu Ser Glu
115 120 125

Lys Tyr Lys Ser Asp Leu Asp Ser Ile Lys Lys Tyr Ile Asn Asp Lys
130 135 140

Gln Gly Glu Asn Glu Lys Tyr Leu Pro Phe Leu Asn Asn Ile Glu Thr
145 150 155 160

Leu Tyr Lys Thr Val Asn Asp Lys Ile Asp Leu Phe Val Ile His Leu
165 170 175

Glu Ala Lys Val Leu Asn Tyr Thr Tyr Glu Lys Ser Asn Val Glu Val
180 185 190

Lys Ile Lys Glu Leu Asn Tyr Leu Lys Thr Ile Gln Asp Lys Leu Ala
195 200 205

Asp Phe Lys Lys Asn Asn Asn Phe Val Gly Ile Ala Asp Leu Ser Thr
210 215 220

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Asp Tyr Asn His Asn Asn Leu Leu Thr Lys Phe Leu Ser Thr Gly Met
225                 230                 235                 240

Val Phe Glu Asn Leu Ala Lys Thr Val Leu Ser Asn Leu Leu Asp Gly
                245                 250                 255

Asn Leu Gln Gly Met Leu Asn Ile Ser Gln His Gln Cys Val Lys Lys
            260                 265                 270

Gln Cys Pro Gln Asn Ser Gly Cys Phe Arg His Leu Asp Glu Arg Glu
        275                 280                 285

Glu Cys Lys Cys Leu Leu Asn Tyr Lys Gln Glu Gly Asp Lys Cys Val
    290                 295                 300

Glu Asn Pro Asn Pro Thr Cys Asn Glu Asn Asn Gly Gly Cys Asp Ala
305                 310                 315                 320

Asp Ala Lys Cys Thr Glu Glu Asp Ser Gly Ser Asn Gly Lys Lys Ile
                325                 330                 335

Thr Cys Glu Cys Thr Lys Pro Asp Ser Tyr Pro Leu Phe Asp Gly Ile
            340                 345                 350

Phe Cys Ser Ser Ser Asn Phe Leu Gly Ile Ser Phe Leu Leu Ile Leu
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Met Leu Ile Leu Tyr Ser Phe Ile
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<210> 35
<211> 114
<212> PRT
<213> Plasmodium falciparum

<400> 35

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Asn Ile Ser Gln His Gln Cys Val Lys Lys Gln Cys Pro Gln Asn Ser
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Gly Cys Phe Arg His Leu Asp Glu Arg Glu Glu Cys Lys Cys Leu Leu
            20                  25                  30

Asn Tyr Lys Gln Glu Gly Asp Lys Cys Val Glu Asn Pro Asn Pro Thr
        35                  40                  45

Cys Asn Glu Asn Asn Gly Gly Cys Asp Ala Asp Ala Lys Cys Thr Glu
    50                  55                  60

Glu Asp Ser Gly Ser Asn Gly Lys Lys Ile Thr Cys Glu Cys Thr Lys
65                  70                  75                  80

Pro Asp Ser Tyr Pro Leu Phe Asp Gly Ile Phe Cys Ser Ser Ser Asn
                85                  90                  95

Phe Leu Gly Ile Ser Phe Leu Leu Ile Leu Met Leu Ile Leu Tyr Ser
            100                 105                 110

Phe Ile
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<210> 36
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 36
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<210> 37
<211> 33
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_feature
<223> Synthetic Oligonucleotide

<400> 37
ccgggatcct tagatccgct gctctttgac ctc 33

<210> 38
<211> 1287
<212> DNA
<213> Plasmodium falciparum

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acaaaaatga aaggaaagat ttgctcaagt tacgtaaaat atatatgttt aacaatatgt 120

gttataggaa tgttatgtat aaaattaagg gataaatatg aaggatatgc tgcttcaggt 180

atacaaaaca ataatgtata tttaagaaat ttatcagagt tacaaaaggg aaatcaacct 240

tgcttgagac atacaaacag aacggataat tcaaagatga acaaagtcaa aaataataat 300

cagacagaaa ataatgacaa caaaaaaaag ctaggtaata aggaagataa ccagggaaaa 360

aataaaaata ataataataa agaaaaacaa aatgacatta ataaaagagg aacacaaaat 420

accgaaacta aaaaaagtaa taaaaaatta agtcaggact ataatgatgt aaataagaaa 480

tttacaaaag aacaaatgaa aaatttagtt aattcattag atgaaattcc accccgaaac 540

gatatggaaa agatatggaa tcatgccgtt aaaacagcta atagtggaac aagcagaatt 600

aaaaaaaaat taaaagaata tgaacaaaaa tatggaagat gctatgaaga gagaccaaat 660

cgttttggat catatgaaca ggtgttaata agccagccac atgaatttaa tgaaagatta 720

aaagttcatg aaaatgatta tactgttttt ttttatgaac tacttgataa agaccctaca 780

cttgatgaaa taaaaaatta tattacttca tttttagaag gttttcaaaa tttgatagac 840

tttcttttta ataaatataa aattatattt ttgcaaacaa ctacggaaat tcctatagac 900

ggaactattt atgataccag taagaaagat atgaagaaaa ataaaaacaa aaagcaaaat 960

ataaaacaag gaggtaaaaa ggaagaggta aaacaagaag gtaaaaagga agaggtaaaa 1020

caagaaggta aaaaggaaga ggtaaaacaa gaaggtaaaa aggaagaggt aaaacaagaa 1080

ggtaaaaagg aagaggtaaa acaaggaggt aaaaaggaag aggtaaaaca aggaggtaaa 1140

aaggaagagg taaaacaagg aggtaaaaag gaagaggtaa aacaaggagg taaaaaggag 1200

gaggtaaaac aaggaggtaa aaaggaggag gtaaaacaag gaggtaaaaa ggaagaggtg 1260

aaaaaagaat taaaaaaaaa caattaa 1287

<210> 39
<211> 3576
<212> DNA
<213> Plasmodium falciparum

<400> 39
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gaaaatttta gtggttcctt taaatgttta ttcaaaaaca agaggaataa atataatgtt 120

gaaatattaa agaatgatta taatacgcta acagaaagtc ataatataat taatagaagg 180

tctagaaatt taggagcgaa tccagaatcc attagtttag gttatgaatt aagtgaaaag 240

gatgaaggaa ataaaaatga tctaataaat agtgctacag atgtatcaac agaattagag 300

aatttaaaag aacgtttatt tcctgaacta gaattatata caaacgatca aaattcaaga 360

aataatactc caaatttacg taagggttct ttgggatttg atagttttaa aaaattggaa 420

ttaggaacac taaatcaatt tgataaagat aaaatgatta atctgaaaga tgaaaccaat 480

atgaatgaat ttgaaggatt tctaggaaga aattcaatgg ctagtaatgt agttacatcc 540

gaattatttg atgaaccagt agatgatagt agtagtacta ctactagcac aggtacaaaa 600

ttgcaaaacg ttccatcgaa tgataataac ggtgaacttt tgaaagatga acctatagat 660

gattatataa ataataattc gaaagttgaa tcggaagata attattatgc acaacagaat 720

atgcaaagtc agtcgaaaga taattatgct tcagaacaaa atgtagcaga tcaatcgaca 780

gataattatc ctacgcaaca tgatgtacca gttcaattga gagacaatta tgcttcagaa 840

caagagtatt ttgatagagg tgaacaattg aatgacgtaa gtgcagataa caatacaagt 900

aataaattga aagacgaacc tgtagataac aatacaagta ataaattgaa agacgaacct 960

gtagataaca atacaagtaa taaattgaaa gacgaacctg tagatgacaa tacaagtaat 1020

aaattgaaag acgaacctgt agataacaat acaattaata aattgaaaga cgaacctgta 1080

gatgacaata caagtaatat tttgaaagac gaacctgtag atgaccatgc aggtaaacat 1140

ttgaaagatg aacctgtaga tgaccatgca ggtaaacata tgaaagatga acccgttgat 1200

attgatagaa caaatattaa aaagggttta aatgaacaac atgttaatcc atggactaca 1260

acattagcag atttaaaaaa tattaataat agtatgaaaa tagaaaaaaa taataaaagt 1320

aatgaacagg taaaaaatac gagcgttagc aaatcatgtg atattataaa accttccaag 1380

tttaataaaa agaacctttt tgagcaaaga cttcaaagtg ttgaaggtaa aaacttttt 1440

gaaggaagaa gtcaaaattt agaaggaaga agtaattttg atgagagatc tcaaattgta 1500

gaacaaagga gaaactttga tgacagggac cagaacataa tggatagaaa aaattttgat 1560

gaaagaaatc aacaggttaa tgacagaaga aattttgatg aaagaaatca acaggttaat 1620

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agaaatcaac aggttaatga cagaagaaat tttgatgaaa gaaatcaaca ggttaatgac 1740

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agaaactttg atgacaggga tcagaacgta atggatagaa gaaactttga tgaaagaaat 1920

caacaggtta atgacagaag aaattttgat gaaagaaatc aacaggttaa tgacagaaga 1980

aattttgatg aaagaaatca acatgttaat gacagaagaa attttgatga aagaaatcaa 2040

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tttgatgaaa gaaatcaaca agttaatgac agaagaaatt ttgatgaaag atatcaaaat 2160

gttaatgaga gaagaaattt tgatgagaga aatcaacaag ttaatgacag aagaaatttt 2220

gatgagagaa atcaacatgt taatgagaga tatcaaaatg ttaatgatag aagaaatttt 2280

gatgaaagaa atcaacaagt taatgacaga agaaattttg atgagagaaa tcaacatgtt 2340

aatgagagaa gaaattttga tgagagaaat caacatgtta atgagagata tcaaaatgtt 2400

aatgatagaa gaaattttga tgaaagaaat caacatgtta atgagagacg aaactttgat 2460

caaagggctc caaatgtaga agagcgaaga tatatggatc caagaaatcc gaatattcca 2520

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ccttgggttc catttatggg agatggaaga ggttataatt tttataatcc tcatcaacat 2640

atggtatatg gaagacccta ttattgggta cctccacccc cagcgttaga atatacaaaa 2700

ggatttaatc caatggaaca gagaagagaa gaagacaggg gacatatggg aggaagaggt 2760

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agagaagaaa ggaataatta taatagtgat tattatacta gaggaaatga gagaacatat 3060

aataattcaa atgtaacaag tagttcaaat agagaattaa taccttacaa aaaagagata 3120

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agtcaatatt tagaaaaaac atatcttgtt cctacagctt ttagaaaaaa atactggtgg 3360

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ttccatcaat tcttacgtaa aggttcttgt gaaaaacgtg aatttttata ttttattaat 3480

tctaaaagaa aaggatgggc tgatcttacg gaaacaatga aaaatatatg gatggaaaga 3540

ttaacttata aaatgagaaa atatagtgga gcataa 3576

<210> 40
<211> 903
<212> DNA
<213> Plasmodium falciparum

<400> 40
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gaacccataa ttcaaaataa aatagttgaa atacccaaag aagtatatct tgaaaagatt 180

gtagaagttc ctcaaataaa aactgtagaa agaatagtgg aacagataag gcccgttatt 240

aagtacaaaa atgtgtataa acccaaaatt gtatatgttg aaaaagtaaa aaatgtagat 300

aaaattatat accaagagaa aattgttgaa gttccacaaa taaaaactgt tgaaaaaatt 360

gtagaagtcc cagtatatgt taacagagaa agaattatta ctgttccaag atatatggtt 420

gtagaaaaag taatacccgt attaaaaaca tccaaaagag aaagtataat ggaagttcca 480

gaagttaatt gcccacacat tgatataagt aaagaagtag aagataaaga agaaatacca 540

attaacgaat taaaagagaa ccaaaccata agtcttgctg atgaaaaaga aatccaaata 600

ttaaatgact taactagcca aaaggtagat tctaatgcaa ccattaatat ggaaggtgaa 660

caagatacaa ctgtagatac tattacacaa gaaaacttct gtggaacagt tagttgtaat 720

ttcttaccaa attatccaaa cttctccaaa attggaaacc cattatgcaa aggaggtcca 780

gaaaaagaaa aacgtttttc aagtatcagc atctacaaat caaaggattc aggattccca 840

agtataagaa ttgcaaaaac tccacaaatg ttccaaagaa atctttactg ttcatatgct 900

taa 903

<210> 41
<211> 1203
<212> DNA
<213> Plasmodium falciparum

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cttactataa tattgtctaa gtttgttgta atccctttga tggctcaaat gtttttgtac 120

acattcatta caatatatat tggaagtcat gatagtttga aacaattaga aattgatgat 180

aaaaccaaaa agtcagacaa cataacagcc tatgatgcta tgatgtttcc agtaattgga 240

tctgcagctt tgcttacttt atatttcgca tataagttct tagatccgtt ttatgtgaat 300

ttattattga ctctttacct aaccttggcg ggtgtatttt ccttacaggg tgtatttaca 360

acaatcttgg aacctgtttt tccaaatttt tttaaaaaag atgaatatgt caaaacattc 420

aaattaccaa attttatata taaagaacct attgtattca atactaataa aggagaaata 480

gtttgcttaa tactcagctt tgctatagga ttgcgttgga tattttataa agacttcatt 540

acacataacg ttttggcagt ttctttttgt tttcaagcca tatctttggt aattcttagc 600

aacttttaa taggattctt attattatct ggtttgtttg tatatgatat tttctgggtt 660

tttggaaacg atgttatggt tacagtagct aagtcttttg aagctccagt aaaattgtta 720

ttcccagttt cgagtgatcc agtacattac agtatgcttg gtttaggaga tattattata 780

ccaggaatat tgatgtcttt atgtttacgt tttgattatt atttatttaa gaataacata 840

cataaaggaa acttaaagaa aatgtttaat gatatatcta tacatgaatc tttcaagaaa 900

tattattttt ataccattat aatattttac gaattaggtt tagttgttac atattgtatg 960

ctcttttatt ttgaacatcc tcaaccagct cttctttatt tggtacctgc atgtatactt 1020

gccatattag cttgttccat atgcaaaaga gaatttaaat taatgataaa atatcaagaa 1080

attacagaca aatccaatac tgtagatgat gcaagtaaga ataaaaaaaa agataaggaa 1140

gaaatcccca aaattcaaga gaccccagtg tcaaatgcaa aaaaaagaat taccaataaa 1200

tga 1203

<210> 42
<211> 3996
<212> DNA
<213> Plasmodium falciparum

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gagaatttga aagaagaaaa ggaaaaaaga ttatataata atttggaata tgtaaattta 120

ttagacataa gaactttgga aaataaatct atatatgtat cttcagattt attgaatttt 180

ttaaaatgct attcaaattt gaatatcaac ttgaataagg ttccttatga tttggtctat 240

tcatttttgc ttgatggaga attatattta ggatatgata tatctgtttt tattttatta 300

gtaaaagcag aacattttga atattgtaga agaatagata atgaaaatag tgataagaaa 360

gaaagtttta gaacaaaaaa taaatcaaca attaaaagat catcacagat agatgatgaa 420

gataatttac aaggattgtt gattaaagaa aaagaagatt atttatcatt tttgaatgaa 480

aataatgagg ctttaaaaca atatatggaa tccgaaaaaa gaggaaatcc tttgtggcat 540

ttggatgaat ctaaatatat ggataaagat tggtatgatg aagaagattc atcatttata 600

tttaagccta cttttaatta tttaggaaag aataataata ataataataa tcataataat 660

aataatgctt tttctaattt tgtaatgggc aacttatctt ctgataatat ttctggatgc 720

ttctttgtgg agaaattaaa tgcttatctt ttcgccatgt tggataaatg tagcaataaa 780

acagttatat ctgtttttcc atatgaaaaa tttggaagac acgaatccag aaatttagct 840

atccaatttt cccaatatga ggactatatg cataggataa ttgaggacag actttatgcg 900

aatattcaaa ataatctccc aagtgttcac aatatgaaga atatgagtaa tatgaataat 960

ataaacaata ataataaaga tattattatt aatagaagtg gtatttctaa tggtaatagc 1020

caaagtgttc cttgctttga aaatattttg gattatgata aattaaaatt tgtggaatat 1080

ataaattcct ttagtgatgt aaagaaatca tcttcattcg atattattgg tagcagcaaa 1140

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gaaagtggat ttgaaaatta tattttggaa aataaacaac cattggaatt aattgaaaat 1260

catttcgaca taatggaaaa cattaaaggg atgtatgata atacaaatca ggaggaaatg 1320

aatttcaata atgtttcagg gttgttgagg gaagacaatt caaatatgaa tgaaatatac 1380

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aatattaaat atattaataa tcattttaat aataaggatg atatgattat gaaatgtaaa 1500

aatatgaagg gatctatttc tatggataat aatagtagta atagtaatag taataatact 1560

cattttgaga aaacattgga atccataaat cctgatgacc ataatatttt taacagtgaa 1620

atggattcta tgaaaaatga aaataacgat gaagaagaac aaacagccac aagtatttat 1680

aacattttag gaaagattgg aaaagataca tatattaaaa gatgtagtag taattataac 1740

tatgataaca ataatggata tagtaacgaa agtagtgaca attataataa tgggtataat 1800

gatagtacag ataataataa tggatataat agtaatagta gctataatag taataataat 1860

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aataaggaca ataataataa tgatggaaat ggtagtagta ataataataa taatgatgat 2040

gatgacgaag aagaagagga tgatgaagat gataacaata ataataatga tgatgataat 2100

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acaaataaga gtgaaattag tactaactat tttgagaaca gttgtaacat gagtgtaaat 2340

aatagtaata acgaagcata tgatgataat tgtaataatg gttttatgaa tcatgacgaa 2400

ggattaactc ttaataatgg gaatgtttca aataataaat gcgatattat aataccagaa 2460

gatggaagtg ttatgtatga aaatatgatt aacagaggaa acggtcttac aagtaacatt 2520

aacaataata ataatgtaag taataataat agtataagtt gcaatgcaga tgataatgta 2580

tataataata taaataatta tataaacaca tatatggaaa ctacaaacaa taagaatcat 2640

attgagaata gatgtaatca agattcatac agtacgaatg aagaaccttt atccaatcat 2700

tctataaatg atccaggaaa aataaaagat ggcataatgt atgatggaaa tgatttggat 2760

atgaatggta cccaagaaca tagtaaagaa gaagggatgg atgtttttga acctaatttt 2820

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aagaaaatta gtaaaaaaag aagtaaagtg aaacatgaaa gaaatagtaa aatacttgat 2940

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cattatttcc ctattagtca atatggattt gaaaatgctc gagaaagagc agttagttgt 3120

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caaataaaag tcaatggtac ttctcacata aaaaaagctg caaaattacc aagatgtgaa 3240

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ttttctattg aagaacttgg attttatgaa gcaagggaaa aagctatata ttgtagaaaa 3360

acatttgaaa aggtaaatgt tcatgatgat tatgaatgtt tattaaatga ccgattaggt 3420

ttacgtaatg aggaaaaaga tgaattatct gatctaatta atatagataa aaatgcattg 3480

gataatctag aactggaaac atctgttcat aataataata aagtgaaaca taataataac 3540

aacaacaaca acaataataa taataataat aataataata ataataattc tgaaaaaatg 3600

agaattaaaa ataatgattt ttcagttgat aataataatg aaaatgttgg aacaggagaa 3660

attaaaatat ccaatgataa atatttaaaa ataacacaag aagctattga aatgattcta 3720

agtaatatca aacataaatc cttaccagaa attaaaatga aattaattga taaacaaaag 3780

tttgaaaatt ataatacatt actagataaa cattttaaat ttattacatc tgtaaaaaac 3840

atttcacagt taagacgata tatatcactc tttcacaaat ttataattta tcatacactt 3900

cctcataata tttctttaag gaaacaatta tttattatcg aagctttaga atggtcttcg 3960

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<211> 876
<212> DNA
<213> Plasmodium falciparum

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gacaaaagga caggagcctt aggttatcta aatctaagtt atggaatggg tattatattc 180

ggtagtttct tagcaggtgt tatggtaaac tttgtaggat caagaggaaa tttattaatt 240

gcattattat cccaattaat agctttatgt ataagtacaa cgttagaaga agatccgaaa 300

ttattgaaga gctctaatgt ggataaaatg aaaatgtcag aaatactttt aagtattaaa 360

aatgaataca taagagtatt aaatttattt aaaaaaacat atggaatatg tttattaata 420

ctttttggat tattacctat attaatgaca aaatttgctt ttgctcctgt ggttgtagat 480

atgttcaaat taactccttc acacacatca tatctaatga cttatgcagg tataataact 540

attattgctg aagggatact tgctccttat ttaagttctt tactagggga tatgatttgt 600

tgtaaatatt cgataccact aacattaaca ggatttttat tattatcatt atgtggcgct 660

aacgaatcac ttgttcttat atttatgtct ataccattat gtggaggtgc tttattatat 720

atatgtggaa ctagccaaat gacaaaacga gtggaagaat cagaattggg ttcgattatt 780

ggtttaaata catctctttt ttatgccgtt acaataatag ctccatatat tgcctttaaa 840

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<210> 44
<211> 2712
<212> DNA
<213> Plasmodium falciparum

<400> 44

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tttttagatt ttgtggatga acctgaacaa ttttactggt tcgtggaaca ttttttgtct 180

gtgaaatttc gagttccaaa gcatcttaaa gataaaaaca ttcataattt tacaccttgc 240

ttaaatagat catgggtatc tgaattttta aaagaatatg aagagccatt tgtaaatcct 300

gttatgaaat ttctagataa agagcaaaga ttatttttta catataactt tggagatgta 360

gaaccacaag gtaaatatac atatttccca gttaaggaat ttcacaaata ttgtatacta 420

ccccccttaa taaaaactaa tataaaagat ggtgaaagtg gagaattttt aaaatatcaa 480

ttaaataaag aagaatataa agtttttctt tcttcggttg gttcccaaat gacagctata 540

aaaaatttat attcaacagt tgaagatgaa caaagaaaac aattattaaa agttatcata 600

gaaaatgaaa gtacaaatga tatatctgtt caatgcccaa cttataacat aaaattacat 660

tatactaaag aatgtgctaa tagtaataat atattaaaat gtattgatga atttcttaga 720

aaaacatgtg aaaagaaaac cgaaagtaaa cacccttctg cagacttatg tgaacactta 780

caatttcttt ttgaatcatt aaagaatcct tacttggata attttaaaaa atttatgact 840

aacagtgatt ttaccttaat caaacctcaa tcagtatgga atgtacctat attcgatata 900

tataaaccaa aaaattattt agatagtgtc caaaatttag atacagaatg ttttaagaaa 960

ttaaatagca aaaatttgat cttcttatca ttccatgatg atatacctaa caatccatat 1020

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aaattgtata atttcttctt cgtttttaaa aaaagtggag ctcccattag tccagtgtca 1140

gttaaagaat tgagccataa tatcaccgat tttagcttta aagaggacaa cagtgaaatt 1200

caatgccaaa atgtaagaaa gagtttagat ttagaagtag atgtagaaac aatgaaaggt 1260

attgcggcag aaaagttatg taagatcatt gaaaaattta ttcttacaaa agatgatgca 1320

agtaaaccag aaaagagtga tatacacaga ggtttccgta tcttatgtat attaatatct 1380

actcatgtgg aagcttataa catagttaga caattattaa atatggaaag tatgatatca 1440

ttaacaagat atacttcatt atatatccat aaatttttta agagtgtaac attattaaaa 1500

ggaaactttt tatataaaaa caataaggct ataagatatt cacgtgcttg tagtaaagcc 1560

tcattacacg ttccatccgt tttatacaga agaaatatat atattcctga aacattctta 1620

tcattatatt taggattatc aaatttagta tcttcaaatc ctagtagtcc atttttgaa 1680

tatgcaatta tagaattttt agtaacttat tacaataagg gttctgaaaa attcgttctt 1740

tattttatat ctattatatc agtattatat atcaacgaat attattatga acaactttca 1800

tgtttctatc caaaagaatt tgaattaata aaatccagaa tgatacatcc aaatatagta 1860

gatcgtatat taaagggtat agataactta atgaaaagta caagatatga taaaatgcgt 1920

acaatgtatt tggatttcga aagttccgat attttctcca gagaaaaagt tttcaccgcc 1980

ttatacaact tcgatagctt cattaagacc aatgaacaat taaagaagaa gaacttagaa 2040

gaaatatcag aaatacctgt acaattagaa acatctaatg atggtattgg atacagaaaa 2100

caagacgttc tttatgaaac tgataaacca caaactatgg atgaagcttc atatgaagaa 2160

actgtagatg aagatgctca ccatgttaat gaaaaacaac acagtgccca cttcttagat 2220

gctattgcgg aaaaagacat attagaagaa aaaaccaagg atcaagattt agaaatagaa 2280

ttatacaaat atatgggacc attaaaagaa caatctaaaa gtacaagtgc tgcatctact 2340

agtgatgaaa tatcaggttc tgaaggtcca tctactgaat ctacaagtac aggaaatcaa 2400

ggtgaagata aaacaacaga taatacatac aaagaaatgg aagaattaga agaagctgaa 2460

ggaacttcaa atcttaaaaa aggtttagaa ttttataaat cttctctaaa acttgatcaa 2520

ttagataaag aaaaacctaa aaagaaaaaa tctaaaagaa aaaaaaagag agacagttct 2580

agtgacagaa tattattaga agaatctaaa acctttactt ctgaaaatga attgatgaga 2640

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tataatttat aa 2712

<210> 45
<211> 2232
<212> DNA
<213> Plasmodium falciparum

<400> 45
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gaaaatttat ttaaatttgt taaatgtgaa tattgtaatg aacatactta tgttaaaggt 180

aagaaagctc cttcagatcc tcaatgtgct gatataaaag aagaatgcaa agaattactt 240

aaggaaaaac aatacacaga ttcagttaca tatttaatgg atggttttaa atcagcaaat 300

aattcagcaa ataatggtaa aaaaaataac gctgaagaaa tgaaaaattt agtaaatttc 360

ttacaatctc ataagaaatt aattaaagca ttaaaaaaga atattgaaag tatacaaaat 420

aagaaacact taatttataa aaacaaatca tataatccat tattactttc ttgtgttaaa 480

aaaatgaata tgttaaaaga aaatgttgac tatattcaaa aaaatcaaaa cttatttaaa 540

gaattaatga atcaaaaagc tacctactct tttgttaata ccaaaaaaaa aattatttct 600

ttaaaatcac aaggtcataa aaaagaaacc tcacaaaatc aaaatgaaaa taacgacaat 660

caaaaatatc aagaagttaa tgatgaagat gatgtaaatg atgaagaaga tacaaacgat 720

gacgaagata ctaacgatga agaagataca aacgatgacg aagatacaaa tgatgacgaa 780

gatactaacg atgaagaaga tactaacgac gaagaagatc atgaaaataa taatgctaca 840

gcatacgaat taggtatcgt cccagttaac gatgtgttaa atgttaatat gaaaaatatg 900

ataacaggaa ataattttat ggatgttgtt aaaaatacat tagctcaatc aggtggatta 960

ggaagtaatg atttaataaa tttcttaaat caaggtaaag aaataggaga aaatttatta 1020

aacataacaa agatgaactt gggagataag aataatcttg aaagttttcc tttagatgaa 1080

ttaaatatgt taaaagataa tttaataaac tatgaattca tattagataa tttgaaaaca 1140

agtgttttaa ataaattaaa agatttatta ttaagattat tatacaaagc atatgtatca 1200

tataagaaaa gaaaagctca agaaaaagga ttaccagaac ctactgttac taatgaagaa 1260

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gttaaaagcc tattaaaaaa attaaaaaat aaaatattcc ctaagaaaaa agaagataat 1380

caagcagtag ataccaaaag tatggaagaa cccaaagtta aagcacaacc agctcttaga 1440

ggtgttgaac caacggaaga ttctaatatt atgaacagta ttaataatgt tatggatgaa 1500

attgatttct ttgaaaaaga attaatcgaa aataataata cacctaatgt tgtaccacca 1560

actcaatcaa aaaaaaaaaa caaaaatgaa actgtatctg gtatggatga aaattttgat 1620

aatcatcctg aaaattattt taaagaagaa tattattatg atgaaaatga tgatatggaa 1680

gtaaaagtta aaaaaatagg tgtcacatta aaaaaatttg aaccacttaa aaatggaaat 1740

gttagtgaaa ccattaaatt gattcattta ggaaataaag ataaaaaaca cattgaagct 1800

ataaacaacg atattcaaat tattaaacaa gaattacaag ctatttataa tgaacttatg 1860

aattatacaa atggaaacaa aaatattcaa caaatatttc aacaaaatat tctagaaaat 1920

gatgttctta atcaagaaac ggaggaagaa atggaaaaac aagttgaagc aatcaccaag 1980

caaatagaag ctgaagtgga tgccctcgca ccaaaaaata aggaagaaga agaaaaagaa 2040

aaggaaaaag aagaaaaaga aaaagaagaa aaagaaaaag aaaaagaaga aaaagaaaaa 2100

gaagaaaaag aaaaagaaga aaaagaaaaa gaagaaaaag aagaagaaaa aaaagaaaaa 2160

gaagaagaac aagaagaaga agaagaagaa gaaatagtac cagaaaattt gacaactgaa 2220

gaatcaaaat aa 2232

<210> 46
<211> 428
<212> PRT
<213> Plasmodium falciparum

<400> 46

```
Met Cys Asn Lys Leu Ser Arg Gly Ser Asn Met Asn Lys Ser Glu Leu
1               5                  10                  15

Gly Asp Arg Ser Thr Lys Met Lys Gly Lys Ile Cys Ser Ser Tyr Val
            20                  25                  30

Lys Tyr Ile Cys Leu Thr Ile Cys Val Ile Gly Met Leu Cys Ile Lys
        35                  40                  45

Leu Arg Asp Lys Tyr Glu Gly Tyr Ala Ala Ser Gly Ile Gln Asn Asn
    50                  55                  60

Asn Val Tyr Leu Arg Asn Leu Ser Glu Leu Gln Lys Gly Asn Gln Pro
65                  70                  75                  80

Cys Leu Arg His Thr Asn Arg Thr Asp Asn Ser Lys Met Asn Lys Val
                85                  90                  95

Lys Asn Asn Asn Gln Thr Glu Asn Asn Asp Asn Lys Lys Lys Leu Gly
            100                 105                 110

Asn Lys Glu Asp Asn Gln Gly Lys Asn Lys Asn Asn Asn Asn Lys Glu
        115                 120                 125

Lys Gln Asn Asp Ile Asn Lys Arg Gly Thr Gln Asn Thr Glu Thr Lys
    130                 135                 140

Lys Ser Asn Lys Lys Leu Ser Gln Asp Tyr Asn Asp Val Asn Lys Lys
145                 150                 155                 160

Phe Thr Lys Glu Gln Met Lys Asn Leu Val Asn Ser Leu Asp Glu Ile
                165                 170                 175

Pro Pro Arg Asn Asp Met Glu Lys Ile Trp Asn His Ala Val Lys Thr
            180                 185                 190

Ala Asn Ser Gly Thr Ser Arg Ile Lys Lys Lys Leu Lys Glu Tyr Glu
        195                 200                 205

Gln Lys Tyr Gly Arg Cys Tyr Glu Glu Arg Pro Asn Arg Phe Gly Ser
    210                 215                 220

Tyr Glu Gln Val Leu Ile Ser Gln Pro His Glu Phe Asn Glu Arg Leu
225                 230                 235                 240

Lys Val His Glu Asn Asp Tyr Thr Val Phe Phe Tyr Glu Leu Leu Asp
                245                 250                 255

Lys Asp Pro Thr Leu Asp Glu Ile Lys Asn Tyr Ile Thr Ser Phe Leu
            260                 265                 270

Glu Gly Phe Gln Asn Leu Ile Asp Phe Leu Phe Asn Lys Tyr Lys Ile
        275                 280                 285

Ile Phe Leu Gln Thr Thr Thr Glu Ile Pro Ile Asp Gly Thr Ile Tyr
    290                 295                 300
```

```
Asp Thr Ser Lys Lys Asp Met Lys Lys Asn Lys Asn Lys Lys Gln Asn
305                 310                 315                 320

Ile Lys Gln Gly Gly Lys Lys Glu Glu Val Lys Gln Glu Gly Lys Lys
                325                 330                 335

Glu Glu Val Lys Gln Glu Gly Lys Lys Glu Glu Val Lys Gln Glu Gly
            340                 345                 350

Lys Lys Glu Glu Val Lys Gln Glu Gly Lys Lys Glu Glu Val Lys Gln
        355                 360                 365

Gly Gly Lys Lys Glu Glu Val Lys Gln Gly Gly Lys Lys Glu Glu Val
    370                 375                 380

Lys Gln Gly Gly Lys Lys Glu Glu Val Lys Gln Gly Gly Lys Lys Glu
385                 390                 395                 400

Glu Val Lys Gln Gly Gly Lys Lys Glu Glu Val Lys Gln Gly Gly Lys
                405                 410                 415

Lys Glu Glu Val Lys Lys Glu Leu Lys Lys Asn Asn
            420                 425
```

<210> 47
<211> 1191
<212> PRT
<213> Plasmodium falciparum

<400> 47

```
Met Ile Phe Val Lys Ser Lys Ile Leu Tyr Phe Leu Lys Trp Pro Ser
1               5                   10                  15

Val Ala Ile Glu Glu Asn Phe Ser Gly Ser Phe Lys Cys Leu Phe Lys
            20                  25                  30

Asn Lys Arg Asn Lys Tyr Asn Val Glu Ile Leu Lys Asn Asp Tyr Asn
        35                  40                  45

Thr Leu Thr Glu Ser His Asn Ile Ile Asn Arg Arg Ser Arg Asn Leu
    50                  55                  60

Gly Ala Asn Pro Glu Ser Ile Ser Leu Gly Tyr Glu Leu Ser Glu Lys
65                  70                  75                  80

Asp Glu Gly Asn Lys Asn Asp Leu Ile Asn Ser Ala Thr Asp Val Ser
                85                  90                  95

Thr Glu Leu Glu Asn Leu Lys Glu Arg Leu Phe Pro Glu Leu Glu Leu
            100                 105                 110

Tyr Thr Asn Asp Gln Asn Ser Arg Asn Asn Thr Pro Asn Leu Arg Lys
        115                 120                 125

Gly Ser Leu Gly Phe Asp Ser Phe Lys Lys Leu Glu Leu Gly Thr Leu
    130                 135                 140

Asn Gln Phe Asp Lys Asp Lys Met Ile Asn Leu Lys Asp Glu Thr Asn
145                 150                 155                 160
```

```
Met Asn Glu Phe Glu Gly Phe Leu Gly Arg Asn Ser Met Ala Ser Asn
                165                 170                 175

Val Val Thr Ser Glu Leu Phe Asp Glu Pro Val Asp Asp Ser Ser Ser
            180                 185                 190

Thr Thr Thr Ser Thr Gly Thr Lys Leu Gln Asn Val Pro Ser Asn Asp
        195                 200                 205

Asn Asn Gly Glu Leu Leu Lys Asp Glu Pro Ile Asp Asp Tyr Ile Asn
    210                 215                 220

Asn Asn Ser Lys Val Glu Ser Glu Asp Asn Tyr Tyr Ala Gln Gln Asn
225                 230                 235                 240

Met Gln Ser Gln Ser Lys Asp Asn Tyr Ala Ser Glu Gln Asn Val Ala
                245                 250                 255

Asp Gln Ser Thr Asp Asn Tyr Pro Thr Gln His Asp Val Pro Val Gln
            260                 265                 270

Leu Arg Asp Asn Tyr Ala Ser Glu Gln Glu Tyr Phe Asp Arg Gly Glu
        275                 280                 285

Gln Leu Asn Asp Val Ser Ala Asp Asn Asn Thr Ser Asn Lys Leu Lys
    290                 295                 300

Asp Glu Pro Val Asp Asn Asn Thr Ser Asn Lys Leu Lys Asp Glu Pro
305                 310                 315                 320

Val Asp Asn Asn Thr Ser Asn Lys Leu Lys Asp Glu Pro Val Asp Asp
                325                 330                 335

Asn Thr Ser Asn Lys Leu Lys Asp Glu Pro Val Asp Asn Asn Thr Ile
            340                 345                 350

Asn Lys Leu Lys Asp Glu Pro Val Asp Asp Asn Thr Ser Asn Ile Leu
        355                 360                 365

Lys Asp Glu Pro Val Asp Asp His Ala Gly Lys His Leu Lys Asp Glu
    370                 375                 380

Pro Val Asp Asp His Ala Gly Lys His Met Lys Asp Glu Pro Val Asp
385                 390                 395                 400

Ile Asp Arg Thr Asn Ile Lys Lys Gly Leu Asn Glu Gln His Val Asn
                405                 410                 415

Pro Trp Thr Thr Thr Leu Ala Asp Leu Lys Asn Ile Asn Asn Ser Met
            420                 425                 430

Lys Ile Glu Lys Asn Asn Lys Ser Asn Glu Gln Val Lys Asn Thr Ser
        435                 440                 445

Val Ser Lys Ser Cys Asp Ile Ile Lys Pro Ser Lys Phe Asn Lys Lys
    450                 455                 460

Asn Leu Phe Glu Gln Arg Leu Gln Ser Val Glu Gly Lys Asn Phe Phe
465                 470                 475                 480
```

Glu Gly Arg Ser Gln Asn Leu Glu Gly Arg Ser Asn Phe Asp Glu Arg
485 490 495

Ser Gln Ile Val Glu Gln Arg Arg Asn Phe Asp Asp Arg Asp Gln Asn
500 505 510

Ile Met Asp Arg Lys Asn Phe Asp Glu Arg Asn Gln Gln Val Asn Asp
515 520 525

Arg Arg Asn Phe Asp Glu Arg Asn Gln Gln Val Asn Asp Arg Arg Asn
530 535 540

Phe Asp Asp Arg Asp Gln Asn Val Met Asp Arg Arg Asn Phe Asp Glu
545 550 555 560

Arg Asn Gln Gln Val Asn Asp Arg Arg Asn Phe Asp Glu Arg Asn Gln
565 570 575

Gln Val Asn Asp Arg Arg Asn Phe Asp Asp Arg Asp Gln Asn Val Met
580 585 590

Asp Arg Arg Asn Phe Asp Glu Arg Asn Gln Gln Val Asn Asp Arg Arg
595 600 605

Asn Phe Asp Glu Arg Asn Gln Gln Val Asn Asp Arg Arg Asn Phe Asp
610 615 620

Asp Arg Asp Gln Asn Val Met Asp Arg Arg Asn Phe Asp Glu Arg Asn
625 630 635 640

Gln Gln Val Asn Asp Arg Arg Asn Phe Asp Glu Arg Asn Gln Gln Val
645 650 655

Asn Asp Arg Arg Asn Phe Asp Glu Arg Asn Gln His Val Asn Asp Arg
660 665 670

Arg Asn Phe Asp Glu Arg Asn Gln Asn Val Asn Asp Arg Arg Asn Phe
675 680 685

Asp Glu Arg Asn Gln Asn Val Asn Asp Arg Arg Asn Phe Asp Glu Arg
690 695 700

Asn Gln Gln Val Asn Asp Arg Arg Asn Phe Asp Glu Arg Tyr Gln Asn
705 710 715 720

Val Asn Glu Arg Arg Asn Phe Asp Glu Arg Asn Gln Gln Val Asn Asp
725 730 735

Arg Arg Asn Phe Asp Glu Arg Asn Gln His Val Asn Glu Arg Tyr Gln
740 745 750

Asn Val Asn Asp Arg Arg Asn Phe Asp Glu Arg Asn Gln Gln Val Asn
755 760 765

Asp Arg Arg Asn Phe Asp Glu Arg Asn Gln His Val Asn Glu Arg Arg
770 775 780

Asn Phe Asp Glu Arg Asn Gln His Val Asn Glu Arg Tyr Gln Asn Val
785 790 795 800

```
Asn Asp Arg Arg Asn Phe Asp Glu Arg Asn Gln His Val Asn Glu Arg
                805                 810                 815

Arg Asn Phe Asp Gln Arg Ala Pro Asn Val Glu Glu Arg Arg Tyr Met
            820                 825                 830

Asp Pro Arg Asn Pro Asn Ile Pro Tyr Val Arg Phe Pro His His Gln
        835                 840                 845

Trp Gly Gln Gly Met Met Tyr Gly Arg Pro Tyr Tyr Pro Trp Val Pro
    850                 855                 860

Phe Met Gly Asp Gly Arg Gly Tyr Asn Phe Tyr Asn Pro His Gln His
865                 870                 875                 880

Met Val Tyr Gly Arg Pro Tyr Tyr Trp Val Pro Pro Pro Pro Ala Leu
                885                 890                 895

Glu Tyr Thr Lys Gly Phe Asn Pro Met Glu Gln Arg Arg Glu Glu Asp
            900                 905                 910

Arg Gly His Met Gly Gly Arg Gly Ser Arg Tyr Pro Glu Glu Glu Arg
        915                 920                 925

Tyr Asn Tyr Asn Asn Lys Arg Ser Asn Ser Ile Pro Glu Gly Arg Asn
    930                 935                 940

Tyr Glu Glu Asn Ala Tyr Glu Arg Gly Gly Gly Asn Asn Lys Trp Asp
945                 950                 955                 960

Phe Arg Asn Met Tyr Asp Arg Leu Arg Asp Glu Asp Glu Asn Asp Tyr
                965                 970                 975

Asp Gln Pro Pro Ser Thr Ser Ser Ser Asn Arg Gly Arg Gly Asn Glu
            980                 985                 990

Arg Tyr Ser Gln Ser Arg Asp Arg  Arg Glu Glu Arg Asn  Asn Tyr Asn
        995                 1000                1005

Ser Asp  Tyr Tyr Thr Arg Gly  Asn Glu Arg Thr Tyr  Asn Asn Ser
    1010                1015                1020

Asn Val  Thr Ser Ser Ser Asn  Arg Glu Leu Ile Pro  Tyr Lys Lys
    1025                1030                1035

Glu Ile  Leu Pro Phe Gly Val  Ser Asn Ser Glu Leu  Glu Asp Lys
    1040                1045                1050

Leu Thr  Glu Glu Glu Leu Asn  Glu Arg Ile Arg Arg  Leu Asp Tyr
    1055                1060                1065

Thr Val  Ser Val Lys Asp Met  Phe Ile Leu Trp Asn  His Ile Leu
    1070                1075                1080

Ala His  Glu Arg Lys Lys Tyr  Thr Lys Met Gln Glu  Tyr Leu Met
    1085                1090                1095

Tyr Tyr  Ser Gln Tyr Leu Glu  Lys Thr Tyr Leu Val  Pro Thr Ala
    1100                1105                1110
```

```
Phe Arg  Lys Lys Tyr Trp Trp  Arg Val His Tyr Met  Leu Thr Glu
    1115                 1120                 1125

Glu Val  Val Lys Arg Glu Arg  Thr Asp Asn Leu Asp  Phe His Gln
    1130                 1135                 1140

Phe Leu  Arg Lys Gly Ser Cys  Glu Lys Arg Glu Phe  Leu Tyr Phe
    1145                 1150                 1155

Ile Asn  Ser Lys Arg Lys Gly  Trp Ala Asp Leu Thr  Glu Thr Met
    1160                 1165                 1170

Lys Asn  Ile Trp Met Glu Arg  Leu Thr Tyr Lys Met  Arg Lys Tyr
    1175                 1180                 1185

Ser Gly  Ala
    1190
```

<210> 48
<211> 300
<212> PRT
<213> Plasmodium falciparum

<400> 48

```
Met Cys Ser Thr Asn Lys Asn Leu Ala Cys Cys Lys Gly Asp Asn Val
1               5                  10                  15

Phe Asp Gly Gln Ile Asn Gly Asn Glu Ser Tyr Pro Gln Val Val Asn
            20                  25                  30

Lys Gln Leu Pro Pro Lys Val Leu Glu Pro Ile Ile Gln Asn Lys Ile
        35                  40                  45

Val Glu Ile Pro Lys Glu Val Tyr Leu Glu Lys Ile Val Glu Val Pro
    50                  55                  60

Gln Ile Lys Thr Val Glu Arg Ile Val Glu Gln Ile Arg Pro Val Ile
65                  70                  75                  80

Lys Tyr Lys Asn Val Tyr Lys Pro Lys Ile Val Tyr Val Glu Lys Val
                85                  90                  95

Lys Asn Val Asp Lys Ile Ile Tyr Gln Glu Lys Ile Val Glu Val Pro
            100                 105                 110

Gln Ile Lys Thr Val Glu Lys Ile Val Glu Val Pro Val Tyr Val Asn
        115                 120                 125

Arg Glu Arg Ile Ile Thr Val Pro Arg Tyr Met Val Val Glu Lys Val
    130                 135                 140

Ile Pro Val Leu Lys Thr Ser Lys Arg Glu Ser Ile Met Glu Val Pro
145                 150                 155                 160

Glu Val Asn Cys Pro His Ile Asp Ile Ser Lys Glu Val Glu Asp Lys
                165                 170                 175

Glu Glu Ile Pro Ile Asn Glu Leu Lys Glu Asn Gln Thr Ile Ser Leu
```

```
            180                 185                 190

Ala Asp Glu Lys Glu Ile Gln Ile Leu Asn Asp Leu Thr Ser Gln Lys
        195                 200                 205

Val Asp Ser Asn Ala Thr Ile Asn Met Glu Gly Glu Gln Asp Thr Thr
    210                 215                 220

Val Asp Thr Ile Thr Gln Glu Asn Phe Cys Gly Thr Val Ser Cys Asn
225                 230                 235                 240

Phe Leu Pro Asn Tyr Pro Asn Phe Ser Lys Ile Gly Asn Pro Leu Cys
                245                 250                 255

Lys Gly Gly Pro Glu Lys Glu Lys Arg Phe Ser Ser Ile Ser Ile Tyr
            260                 265                 270

Lys Ser Lys Asp Ser Gly Phe Pro Ser Ile Arg Ile Ala Lys Thr Pro
        275                 280                 285

Gln Met Phe Gln Arg Asn Leu Tyr Cys Ser Tyr Ala
    290                 295                 300

<210>  49
<211>  400
<212>  PRT
<213>  Plasmodium falciparum

<400>  49

Met Lys Asn Glu Asn Met Gly Asn Ser Ile Phe Tyr Tyr Ser Cys Tyr
1               5                   10                  15

Val Ile Ile Val Leu Thr Ile Ile Leu Ser Lys Phe Val Val Ile Pro
            20                  25                  30

Leu Met Ala Gln Met Phe Leu Tyr Thr Phe Ile Thr Ile Tyr Ile Gly
        35                  40                  45

Ser His Asp Ser Leu Lys Gln Leu Glu Ile Asp Asp Lys Thr Lys Lys
    50                  55                  60

Ser Asp Asn Ile Thr Ala Tyr Asp Ala Met Met Phe Pro Val Ile Gly
65                  70                  75                  80

Ser Ala Ala Leu Leu Thr Leu Tyr Phe Ala Tyr Lys Phe Leu Asp Pro
                85                  90                  95

Phe Tyr Val Asn Leu Leu Leu Thr Leu Tyr Leu Thr Leu Ala Gly Val
            100                 105                 110

Phe Ser Leu Gln Gly Val Phe Thr Thr Ile Leu Glu Pro Val Phe Pro
        115                 120                 125

Asn Phe Phe Lys Lys Asp Glu Tyr Val Lys Thr Phe Lys Leu Pro Asn
    130                 135                 140

Phe Ile Tyr Lys Glu Pro Ile Val Phe Asn Thr Asn Lys Gly Glu Ile
145                 150                 155                 160
```

Val Cys Leu Ile Leu Ser Phe Ala Ile Gly Leu Arg Trp Ile Phe Tyr
165 170 175

Lys Asp Phe Ile Thr His Asn Val Leu Ala Val Ser Phe Cys Phe Gln
180 185 190

Ala Ile Ser Leu Val Ile Leu Ser Asn Phe Leu Ile Gly Phe Leu Leu
195 200 205

Leu Ser Gly Leu Phe Val Tyr Asp Ile Phe Trp Val Phe Gly Asn Asp
210 215 220

Val Met Val Thr Val Ala Lys Ser Phe Glu Ala Pro Val Lys Leu Leu
225 230 235 240

Phe Pro Val Ser Ser Asp Pro Val His Tyr Ser Met Leu Gly Leu Gly
245 250 255

Asp Ile Ile Ile Pro Gly Ile Leu Met Ser Leu Cys Leu Arg Phe Asp
260 265 270

Tyr Tyr Leu Phe Lys Asn Asn Ile His Lys Gly Asn Leu Lys Lys Met
275 280 285

Phe Asn Asp Ile Ser Ile His Glu Ser Phe Lys Lys Tyr Tyr Phe Tyr
290 295 300

Thr Ile Ile Ile Phe Tyr Glu Leu Gly Leu Val Val Thr Tyr Cys Met
305 310 315 320

Leu Phe Tyr Phe Glu His Pro Gln Pro Ala Leu Leu Tyr Leu Val Pro
325 330 335

Ala Cys Ile Leu Ala Ile Leu Ala Cys Ser Ile Cys Lys Arg Glu Phe
340 345 350

Lys Leu Met Ile Lys Tyr Gln Glu Ile Thr Asp Lys Ser Asn Thr Val
355 360 365

Asp Asp Ala Ser Lys Asn Lys Lys Lys Asp Lys Glu Glu Ile Pro Lys
370 375 380

Ile Gln Glu Thr Pro Val Ser Asn Ala Lys Lys Arg Ile Thr Asn Lys
385 390 395 400

<210> 50
<211> 1331
<212> PRT
<213> Plasmodium falciparum

<400> 50

Met Val Leu Val Val Glu Tyr His Asn Ile Asn Thr Pro Val Gly Lys
1 5 10 15

Tyr Ser Glu Leu Glu Asn Leu Lys Glu Glu Lys Glu Lys Arg Leu Tyr
20 25 30

Asn Asn Leu Glu Tyr Val Asn Leu Leu Asp Ile Arg Thr Leu Glu Asn
35 40 45

```
Lys Ser Ile Tyr Val Ser Ser Asp Leu Leu Asn Phe Leu Lys Cys Tyr
    50                  55                  60

Ser Asn Leu Asn Ile Asn Leu Asn Lys Val Pro Tyr Asp Leu Val Tyr
65                  70                  75                  80

Ser Phe Leu Leu Asp Gly Glu Leu Tyr Leu Gly Tyr Asp Ile Ser Val
                85                  90                  95

Phe Ile Leu Leu Val Lys Ala Glu His Phe Glu Tyr Cys Arg Arg Ile
            100                 105                 110

Asp Asn Glu Asn Ser Asp Lys Lys Glu Ser Phe Arg Thr Lys Asn Lys
        115                 120                 125

Ser Thr Ile Lys Arg Ser Ser Gln Ile Asp Asp Glu Asp Asn Leu Gln
    130                 135                 140

Gly Leu Leu Ile Lys Glu Lys Glu Asp Tyr Leu Ser Phe Leu Asn Glu
145                 150                 155                 160

Asn Asn Glu Ala Leu Lys Gln Tyr Met Glu Ser Glu Lys Arg Gly Asn
                165                 170                 175

Pro Leu Trp His Leu Asp Glu Ser Lys Tyr Met Asp Lys Asp Trp Tyr
            180                 185                 190

Asp Glu Glu Asp Ser Ser Phe Ile Phe Lys Pro Thr Phe Asn Tyr Leu
        195                 200                 205

Gly Lys Asn Asn Asn Asn Asn Asn Asn His Asn Asn Asn Asn Ala Phe
    210                 215                 220

Ser Asn Phe Val Met Gly Asn Leu Ser Ser Asp Asn Ile Ser Gly Cys
225                 230                 235                 240

Phe Phe Val Glu Lys Leu Asn Ala Tyr Leu Phe Ala Met Leu Asp Lys
                245                 250                 255

Cys Ser Asn Lys Thr Val Ile Ser Val Phe Pro Tyr Glu Lys Phe Gly
            260                 265                 270

Arg His Glu Ser Arg Asn Leu Ala Ile Gln Phe Ser Gln Tyr Glu Asp
        275                 280                 285

Tyr Met His Arg Ile Ile Glu Asp Arg Leu Tyr Ala Asn Ile Gln Asn
    290                 295                 300

Asn Leu Pro Ser Val His Asn Met Lys Asn Met Ser Asn Met Asn Asn
305                 310                 315                 320

Ile Asn Asn Asn Asn Lys Asp Ile Ile Ile Asn Arg Ser Gly Ile Ser
                325                 330                 335

Asn Gly Asn Ser Gln Ser Val Pro Cys Phe Glu Asn Ile Leu Asp Tyr
            340                 345                 350

Asp Lys Leu Lys Phe Val Glu Tyr Ile Asn Ser Phe Ser Asp Val Lys
        355                 360                 365
```

```
Lys Ser Ser Ser Phe Asp Ile Ile Gly Ser Ser Lys Asn Ile Tyr Glu
    370                 375                 380

Gln Gly Glu Asn Leu Lys Asn Tyr Cys Ile Tyr His Asn Asn Asn Phe
385                 390                 395                 400

Glu Ser Gly Phe Glu Asn Tyr Ile Leu Glu Asn Lys Gln Pro Leu Glu
                405                 410                 415

Leu Ile Glu Asn His Phe Asp Ile Met Glu Asn Ile Lys Gly Met Tyr
            420                 425                 430

Asp Asn Thr Asn Gln Glu Glu Met Asn Phe Asn Asn Val Ser Gly Leu
        435                 440                 445

Leu Arg Glu Asp Asn Ser Asn Met Asn Glu Ile Tyr Leu Thr Arg Asp
    450                 455                 460

Asn His Asn Asn Asn Tyr His Glu Asn Glu Glu Asn Ile Tyr Ser Ile
465                 470                 475                 480

Asn Ile Lys Tyr Ile Asn Asn His Phe Asn Asn Lys Asp Asp Met Ile
                485                 490                 495

Met Lys Cys Lys Asn Met Lys Gly Ser Ile Ser Met Asp Asn Asn Ser
            500                 505                 510

Ser Asn Ser Asn Ser Asn Asn Thr His Phe Glu Lys Thr Leu Glu Ser
        515                 520                 525

Ile Asn Pro Asp Asp His Asn Ile Phe Asn Ser Glu Met Asp Ser Met
    530                 535                 540

Lys Asn Glu Asn Asn Asp Glu Glu Glu Gln Thr Ala Thr Ser Ile Tyr
545                 550                 555                 560

Asn Ile Leu Gly Lys Ile Gly Lys Asp Thr Tyr Ile Lys Arg Cys Ser
                565                 570                 575

Ser Asn Tyr Asn Tyr Asp Asn Asn Asn Gly Tyr Ser Asn Glu Ser Ser
            580                 585                 590

Asp Asn Tyr Asn Asn Gly Tyr Asn Asp Ser Thr Asp Asn Asn Asn Gly
        595                 600                 605

Tyr Asn Ser Asn Ser Ser Tyr Asn Ser Asn Asn Asn Glu Asp Asp Asn
    610                 615                 620

Asn Asn Asn Asn Asn Asn Asp Glu Asn Cys Asp Asn Asn Asn Asn His
625                 630                 635                 640

Asn Asn Asn Asn Tyr Asn Asn Asn Asn Asn Tyr Gly Asn Asn Asn Asn
                645                 650                 655

Asn Asn Asn Asn Asn Lys Asp Asn Asn Asn Asn Asp Gly Asn Gly Ser
            660                 665                 670

Ser Asn Asn Asn Asn Asn Asp Asp Asp Asp Glu Glu Glu Glu Asp Asp
        675                 680                 685
```

```
Glu Asp Asp Asn Asn Asn Asn Asn Asp Asp Asp Asn Met Ser Asp Asn
    690                 695                 700

Glu Glu Met Glu Asp Asn Asp Glu Asp Asn Asp Glu Tyr Asn Asn Ser
705                 710                 715                 720

Asn Asp Ser Tyr Lys Tyr Glu Glu Lys Asp Ser Asn His Glu Lys Asp
                725                 730                 735

Leu Lys Lys Asp Ile Ile Glu Gly Asp Met Ile Asn Ser Val Lys Tyr
            740                 745                 750

Asp Lys Asn Ile Gly His His Thr Thr Asn Lys Ser Glu Ile Ser Thr
        755                 760                 765

Asn Tyr Phe Glu Asn Ser Cys Asn Met Ser Val Asn Asn Ser Asn Asn
    770                 775                 780

Glu Ala Tyr Asp Asp Asn Cys Asn Asn Gly Phe Met Asn His Asp Glu
785                 790                 795                 800

Gly Leu Thr Leu Asn Asn Gly Asn Val Ser Asn Asn Lys Cys Asp Ile
                805                 810                 815

Ile Ile Pro Glu Asp Gly Ser Val Met Tyr Glu Asn Met Ile Asn Arg
            820                 825                 830

Gly Asn Gly Leu Thr Ser Asn Ile Asn Asn Asn Asn Asn Val Ser Asn
        835                 840                 845

Asn Asn Ser Ile Ser Cys Asn Ala Asp Asp Asn Val Tyr Asn Asn Ile
    850                 855                 860

Asn Asn Tyr Ile Asn Thr Tyr Met Glu Thr Thr Asn Asn Lys Asn His
865                 870                 875                 880

Ile Glu Asn Arg Cys Asn Gln Asp Ser Tyr Ser Thr Asn Glu Glu Pro
                885                 890                 895

Leu Ser Asn His Ser Ile Asn Asp Pro Gly Lys Ile Lys Asp Gly Ile
            900                 905                 910

Met Tyr Asp Gly Asn Asp Leu Asp Met Asn Gly Thr Gln Glu His Ser
        915                 920                 925

Lys Glu Glu Gly Met Asp Val Phe Glu Pro Asn Phe Phe Glu Leu Lys
    930                 935                 940

Arg Asn Ser Ser Asp Gly Gln Asn Lys His Leu Glu Pro Gly Val Gln
945                 950                 955                 960

Lys Lys Ile Ser Lys Lys Arg Ser Lys Val Lys His Glu Arg Asn Ser
                965                 970                 975

Lys Ile Leu Asp Asp Glu Lys Lys Glu Val Leu Asn Lys Val Ser Gln
            980                 985                 990

Ile Thr Arg Val Gly Gly Val Cys  Phe Asp Lys Asn Arg  Gln Arg Trp
        995                 1000                1005
```

```
Ile Ala  His Trp Lys Ile Asp  Gly Lys Tyr His Lys  His Tyr Phe
    1010                 1015                 1020

Pro Ile  Ser Gln Tyr Gly Phe  Glu Asn Ala Arg Glu  Arg Ala Val
    1025                 1030                 1035

Ser Cys  Arg Lys Gln Ala Glu  Lys Leu Phe Asn Leu  Pro Glu Ile
    1040                 1045                 1050

Gln Pro  Arg Asn Arg Trp Asn  Gln Ile Lys Val Asn  Gly Thr Ser
    1055                 1060                 1065

His Ile  Lys Lys Ala Ala Lys  Leu Pro Arg Cys Glu  Gly Ile Gly
    1070                 1075                 1080

Tyr Asp  Glu Leu Ser Gln Ser  Trp Val Ser Thr Phe  Val Val His
    1085                 1090                 1095

Lys Lys  Phe Ser Ile Glu Glu  Leu Gly Phe Tyr Glu  Ala Arg Glu
    1100                 1105                 1110

Lys Ala  Ile Tyr Cys Arg Lys  Thr Phe Glu Lys Val  Asn Val His
    1115                 1120                 1125

Asp Asp  Tyr Glu Cys Leu Leu  Asn Asp Arg Leu Gly  Leu Arg Asn
    1130                 1135                 1140

Glu Glu  Lys Asp Glu Leu Ser  Asp Leu Ile Asn Ile  Asp Lys Asn
    1145                 1150                 1155

Ala Leu  Asp Asn Leu Glu Leu  Glu Thr Ser Val His  Asn Asn Asn
    1160                 1165                 1170

Lys Val  Lys His Asn Asn Asn  Asn Asn Asn Asn Asn  Asn Asn Asn
    1175                 1180                 1185

Asn Asn  Asn Asn Asn Asn Asn  Asn Ser Glu Lys Met  Arg Ile Lys
    1190                 1195                 1200

Asn Asn  Asp Phe Ser Val Asp  Asn Asn Asn Glu Asn  Val Gly Thr
    1205                 1210                 1215

Gly Glu  Ile Lys Ile Ser Asn  Asp Lys Tyr Leu Lys  Ile Thr Gln
    1220                 1225                 1230

Glu Ala  Ile Glu Met Ile Leu  Ser Asn Ile Lys His  Lys Ser Leu
    1235                 1240                 1245

Pro Glu  Ile Lys Met Lys Leu  Ile Asp Lys Gln Lys  Phe Glu Asn
    1250                 1255                 1260

Tyr Asn  Thr Leu Leu Asp Lys  His Phe Lys Phe Ile  Thr Ser Val
    1265                 1270                 1275

Lys Asn  Ile Ser Gln Leu Arg  Arg Tyr Ile Ser Leu  Phe His Lys
    1280                 1285                 1290

Phe Ile  Ile Tyr His Thr Leu  Pro His Asn Ile Ser  Leu Arg Lys
    1295                 1300                 1305
```

Gln Leu Phe Ile Ile Glu Ala Leu Glu Trp Ser Ser Phe Phe Ser
1310 1315 1320

Gly Ala Ala Ser Glu Lys Val Glu
1325 1330

<210> 51
<211> 291
<212> PRT
<213> Plasmodium falciparum

<400> 51

Met Glu Val Thr Ser Thr Leu Leu Glu Lys Gly Lys Asn Phe Ala Gln
1 5 10 15

Asp Pro Ser Glu Val Phe Pro Glu Ser Lys Lys Phe Phe Phe Ser Ser
20 25 30

Ile Val Cys Leu Lys Thr Asn Phe Asp Lys Arg Thr Gly Ala Leu Gly
35 40 45

Tyr Leu Asn Leu Ser Tyr Gly Met Gly Ile Ile Phe Gly Ser Phe Leu
50 55 60

Ala Gly Val Met Val Asn Phe Val Gly Ser Arg Gly Asn Leu Leu Ile
65 70 75 80

Ala Leu Leu Ser Gln Leu Ile Ala Leu Cys Ile Ser Thr Thr Leu Glu
85 90 95

Glu Asp Pro Lys Leu Leu Lys Ser Ser Asn Val Asp Lys Met Lys Met
100 105 110

Ser Glu Ile Leu Leu Ser Ile Lys Asn Glu Tyr Ile Arg Val Leu Asn
115 120 125

Leu Phe Lys Lys Thr Tyr Gly Ile Cys Leu Leu Ile Leu Phe Gly Leu
130 135 140

Leu Pro Ile Leu Met Thr Lys Phe Ala Phe Ala Pro Val Val Val Asp
145 150 155 160

Met Phe Lys Leu Thr Pro Ser His Thr Ser Tyr Leu Met Thr Tyr Ala
165 170 175

Gly Ile Ile Thr Ile Ile Ala Glu Gly Ile Leu Ala Pro Tyr Leu Ser
180 185 190

Ser Leu Leu Gly Asp Met Ile Cys Cys Lys Tyr Ser Ile Pro Leu Thr
195 200 205

Leu Thr Gly Phe Leu Leu Leu Ser Leu Cys Gly Ala Asn Glu Ser Leu
210 215 220

Val Leu Ile Phe Met Ser Ile Pro Leu Cys Gly Gly Ala Leu Leu Tyr
225 230 235 240

Ile Cys Gly Thr Ser Gln Met Thr Lys Arg Val Glu Glu Ser Glu Leu

245 250 255

Gly Ser Ile Ile Gly Leu Asn Thr Ser Leu Phe Tyr Ala Val Thr Ile
260 265 270

Ile Ala Pro Tyr Ile Ala Phe Lys Ser Tyr Ile Ala Leu Gly Leu Gly
275 280 285

Leu Tyr Trp
290

<210> 52
<211> 903
<212> PRT
<213> Plasmodium falciparum

<400> 52

Met Arg Ile Trp Gly Lys Asp Val Phe Ala Gly Phe Val Thr Lys Lys
1 5 10 15

Leu Lys Thr Leu Leu Asp Cys Asn Phe Ala Leu Tyr Tyr Asn Phe Lys
20 25 30

Gly Asn Gly Pro Asp Ala Gly Ser Phe Leu Asp Phe Val Asp Glu Pro
35 40 45

Glu Gln Phe Tyr Trp Phe Val Glu His Phe Leu Ser Val Lys Phe Arg
50 55 60

Val Pro Lys His Leu Lys Asp Lys Asn Ile His Asn Phe Thr Pro Cys
65 70 75 80

Leu Asn Arg Ser Trp Val Ser Glu Phe Leu Lys Glu Tyr Glu Glu Pro
85 90 95

Phe Val Asn Pro Val Met Lys Phe Leu Asp Lys Glu Gln Arg Leu Phe
100 105 110

Phe Thr Tyr Asn Phe Gly Asp Val Glu Pro Gln Gly Lys Tyr Thr Tyr
115 120 125

Phe Pro Val Lys Glu Phe His Lys Tyr Cys Ile Leu Pro Pro Leu Ile
130 135 140

Lys Thr Asn Ile Lys Asp Gly Glu Ser Gly Glu Phe Leu Lys Tyr Gln
145 150 155 160

Leu Asn Lys Glu Glu Tyr Lys Val Phe Leu Ser Ser Val Gly Ser Gln
165 170 175

Met Thr Ala Ile Lys Asn Leu Tyr Ser Thr Val Glu Asp Glu Gln Arg
180 185 190

Lys Gln Leu Leu Lys Val Ile Ile Glu Asn Glu Ser Thr Asn Asp Ile
195 200 205

Ser Val Gln Cys Pro Thr Tyr Asn Ile Lys Leu His Tyr Thr Lys Glu
210 215 220

```
Cys Ala Asn Ser Asn Asn Ile Leu Lys Cys Ile Asp Glu Phe Leu Arg
225                 230                 235                 240

Lys Thr Cys Glu Lys Lys Thr Glu Ser Lys His Pro Ser Ala Asp Leu
                245                 250                 255

Cys Glu His Leu Gln Phe Leu Phe Glu Ser Leu Lys Asn Pro Tyr Leu
            260                 265                 270

Asp Asn Phe Lys Lys Phe Met Thr Asn Ser Asp Phe Thr Leu Ile Lys
        275                 280                 285

Pro Gln Ser Val Trp Asn Val Pro Ile Phe Asp Ile Tyr Lys Pro Lys
    290                 295                 300

Asn Tyr Leu Asp Ser Val Gln Asn Leu Asp Thr Glu Cys Phe Lys Lys
305                 310                 315                 320

Leu Asn Ser Lys Asn Leu Ile Phe Leu Ser Phe His Asp Asp Ile Pro
                325                 330                 335

Asn Asn Pro Tyr Tyr Asn Val Glu Leu Gln Glu Ile Val Lys Leu Ser
            340                 345                 350

Thr Tyr Thr Tyr Ser Ile Phe Asp Lys Leu Tyr Asn Phe Phe Phe Val
        355                 360                 365

Phe Lys Lys Ser Gly Ala Pro Ile Ser Pro Val Ser Val Lys Glu Leu
    370                 375                 380

Ser His Asn Ile Thr Asp Phe Ser Phe Lys Glu Asp Asn Ser Glu Ile
385                 390                 395                 400

Gln Cys Gln Asn Val Arg Lys Ser Leu Asp Leu Glu Val Asp Val Glu
                405                 410                 415

Thr Met Lys Gly Ile Ala Ala Glu Lys Leu Cys Lys Ile Ile Glu Lys
            420                 425                 430

Phe Ile Leu Thr Lys Asp Asp Ala Ser Lys Pro Glu Lys Ser Asp Ile
        435                 440                 445

His Arg Gly Phe Arg Ile Leu Cys Ile Leu Ile Ser Thr His Val Glu
    450                 455                 460

Ala Tyr Asn Ile Val Arg Gln Leu Leu Asn Met Glu Ser Met Ile Ser
465                 470                 475                 480

Leu Thr Arg Tyr Thr Ser Leu Tyr Ile His Lys Phe Phe Lys Ser Val
                485                 490                 495

Thr Leu Leu Lys Gly Asn Phe Leu Tyr Lys Asn Asn Lys Ala Ile Arg
            500                 505                 510

Tyr Ser Arg Ala Cys Ser Lys Ala Ser Leu His Val Pro Ser Val Leu
        515                 520                 525

Tyr Arg Arg Asn Ile Tyr Ile Pro Glu Thr Phe Leu Ser Leu Tyr Leu
    530                 535                 540
```

```
Gly Leu Ser Asn Leu Val Ser Ser Asn Pro Ser Ser Pro Phe Phe Glu
545                 550                 555                 560

Tyr Ala Ile Ile Glu Phe Leu Val Thr Tyr Tyr Asn Lys Gly Ser Glu
                565                 570                 575

Lys Phe Val Leu Tyr Phe Ile Ser Ile Ile Ser Val Leu Tyr Ile Asn
            580                 585                 590

Glu Tyr Tyr Tyr Glu Gln Leu Ser Cys Phe Tyr Pro Lys Glu Phe Glu
        595                 600                 605

Leu Ile Lys Ser Arg Met Ile His Pro Asn Ile Val Asp Arg Ile Leu
    610                 615                 620

Lys Gly Ile Asp Asn Leu Met Lys Ser Thr Arg Tyr Asp Lys Met Arg
625                 630                 635                 640

Thr Met Tyr Leu Asp Phe Glu Ser Ser Asp Ile Phe Ser Arg Glu Lys
                645                 650                 655

Val Phe Thr Ala Leu Tyr Asn Phe Asp Ser Phe Ile Lys Thr Asn Glu
            660                 665                 670

Gln Leu Lys Lys Lys Asn Leu Glu Glu Ile Ser Glu Ile Pro Val Gln
        675                 680                 685

Leu Glu Thr Ser Asn Asp Gly Ile Gly Tyr Arg Lys Gln Asp Val Leu
    690                 695                 700

Tyr Glu Thr Asp Lys Pro Gln Thr Met Asp Glu Ala Ser Tyr Glu Glu
705                 710                 715                 720

Thr Val Asp Glu Asp Ala His His Val Asn Glu Lys Gln His Ser Ala
                725                 730                 735

His Phe Leu Asp Ala Ile Ala Glu Lys Asp Ile Leu Glu Glu Lys Thr
            740                 745                 750

Lys Asp Gln Asp Leu Glu Ile Glu Leu Tyr Lys Tyr Met Gly Pro Leu
        755                 760                 765

Lys Glu Gln Ser Lys Ser Thr Ser Ala Ala Ser Thr Ser Asp Glu Ile
    770                 775                 780

Ser Gly Ser Glu Gly Pro Ser Thr Glu Ser Thr Ser Thr Gly Asn Gln
785                 790                 795                 800

Gly Glu Asp Lys Thr Thr Asp Asn Thr Tyr Lys Glu Met Glu Glu Leu
                805                 810                 815

Glu Glu Ala Glu Gly Thr Ser Asn Leu Lys Lys Gly Leu Glu Phe Tyr
            820                 825                 830

Lys Ser Ser Leu Lys Leu Asp Gln Leu Asp Lys Glu Lys Pro Lys Lys
        835                 840                 845

Lys Lys Ser Lys Arg Lys Lys Lys Arg Asp Ser Ser Ser Asp Arg Ile
    850                 855                 860
```

```
Leu Leu Glu Glu Ser Lys Thr Phe Thr Ser Glu Asn Glu Leu Met Arg
865                 870                 875                 880

Lys Lys Lys Lys Lys Lys Lys Lys Lys Asn Asn Asn Glu Ile Lys Asn
                885                 890                 895

Ile Arg Ile Tyr Tyr Asn Leu
            900
```

<210> 53
<211> 743
<212> PRT
<213> Plasmodium falciparum

<400> 53

```
Met Met Asn Met Lys Ile Val Leu Phe Ser Leu Leu Leu Phe Val Ile
1               5                   10                  15

Arg Trp Asn Ile Ile Ser Cys Asn Lys Asn Asp Lys Asn Gln Gly Val
            20                  25                  30

Asp Met Asn Val Leu Asn Asn Tyr Glu Asn Leu Phe Lys Phe Val Lys
        35                  40                  45

Cys Glu Tyr Cys Asn Glu His Thr Tyr Val Lys Gly Lys Lys Ala Pro
    50                  55                  60

Ser Asp Pro Gln Cys Ala Asp Ile Lys Glu Glu Cys Lys Glu Leu Leu
65                  70                  75                  80

Lys Glu Lys Gln Tyr Thr Asp Ser Val Thr Tyr Leu Met Asp Gly Phe
                85                  90                  95

Lys Ser Ala Asn Asn Ser Ala Asn Asn Gly Lys Lys Asn Asn Ala Glu
            100                 105                 110

Glu Met Lys Asn Leu Val Asn Phe Leu Gln Ser His Lys Lys Leu Ile
        115                 120                 125

Lys Ala Leu Lys Lys Asn Ile Glu Ser Ile Gln Asn Lys Lys His Leu
    130                 135                 140

Ile Tyr Lys Asn Lys Ser Tyr Asn Pro Leu Leu Leu Ser Cys Val Lys
145                 150                 155                 160

Lys Met Asn Met Leu Lys Glu Asn Val Asp Tyr Ile Gln Lys Asn Gln
                165                 170                 175

Asn Leu Phe Lys Glu Leu Met Asn Gln Lys Ala Thr Tyr Ser Phe Val
            180                 185                 190

Asn Thr Lys Lys Lys Ile Ile Ser Leu Lys Ser Gln Gly His Lys Lys
        195                 200                 205

Glu Thr Ser Gln Asn Gln Asn Glu Asn Asn Asp Asn Gln Lys Tyr Gln
    210                 215                 220

Glu Val Asn Asp Glu Asp Asp Val Asn Asp Glu Glu Asp Thr Asn Asp
225                 230                 235                 240
```

```
Asp Glu Asp Thr Asn Asp Glu Glu Asp Thr Asn Asp Asp Glu Asp Thr
                245                 250                 255

Asn Asp Asp Glu Asp Thr Asn Asp Glu Glu Asp Thr Asn Asp Glu Glu
            260                 265                 270

Asp His Glu Asn Asn Asn Ala Thr Ala Tyr Glu Leu Gly Ile Val Pro
        275                 280                 285

Val Asn Asp Val Leu Asn Val Asn Met Lys Asn Met Ile Thr Gly Asn
    290                 295                 300

Asn Phe Met Asp Val Val Lys Asn Thr Leu Ala Gln Ser Gly Gly Leu
305                 310                 315                 320

Gly Ser Asn Asp Leu Ile Asn Phe Leu Asn Gln Gly Lys Glu Ile Gly
                325                 330                 335

Glu Asn Leu Leu Asn Ile Thr Lys Met Asn Leu Gly Asp Lys Asn Asn
            340                 345                 350

Leu Glu Ser Phe Pro Leu Asp Glu Leu Asn Met Leu Lys Asp Asn Leu
        355                 360                 365

Ile Asn Tyr Glu Phe Ile Leu Asp Asn Leu Lys Thr Ser Val Leu Asn
    370                 375                 380

Lys Leu Lys Asp Leu Leu Leu Arg Leu Leu Tyr Lys Ala Tyr Val Ser
385                 390                 395                 400

Tyr Lys Lys Arg Lys Ala Gln Glu Lys Gly Leu Pro Glu Pro Thr Val
                405                 410                 415

Thr Asn Glu Glu Tyr Val Glu Glu Leu Lys Lys Gly Ile Leu Asp Met
            420                 425                 430

Gly Ile Lys Leu Leu Phe Ser Lys Val Lys Ser Leu Leu Lys Lys Leu
        435                 440                 445

Lys Asn Lys Ile Phe Pro Lys Lys Lys Glu Asp Asn Gln Ala Val Asp
    450                 455                 460

Thr Lys Ser Met Glu Glu Pro Lys Val Lys Ala Gln Pro Ala Leu Arg
465                 470                 475                 480

Gly Val Glu Pro Thr Glu Asp Ser Asn Ile Met Asn Ser Ile Asn Asn
                485                 490                 495

Val Met Asp Glu Ile Asp Phe Phe Glu Lys Glu Leu Ile Glu Asn Asn
            500                 505                 510

Asn Thr Pro Asn Val Val Pro Pro Thr Gln Ser Lys Lys Lys Asn Lys
        515                 520                 525

Asn Glu Thr Val Ser Gly Met Asp Glu Asn Phe Asp Asn His Pro Glu
    530                 535                 540

Asn Tyr Phe Lys Glu Glu Tyr Tyr Tyr Asp Glu Asn Asp Asp Met Glu
545                 550                 555                 560
```

Val Lys Val Lys Lys Ile Gly Val Thr Leu Lys Lys Phe Glu Pro Leu
565 570 575

Lys Asn Gly Asn Val Ser Glu Thr Ile Lys Leu Ile His Leu Gly Asn
580 585 590

Lys Asp Lys Lys His Ile Glu Ala Ile Asn Asn Asp Ile Gln Ile Ile
595 600 605

Lys Gln Glu Leu Gln Ala Ile Tyr Asn Glu Leu Met Asn Tyr Thr Asn
610 615 620

Gly Asn Lys Asn Ile Gln Gln Ile Phe Gln Gln Asn Ile Leu Glu Asn
625 630 635 640

Asp Val Leu Asn Gln Glu Thr Glu Glu Glu Met Glu Lys Gln Val Glu
645 650 655

Ala Ile Thr Lys Gln Ile Glu Ala Glu Val Asp Ala Leu Ala Pro Lys
660 665 670

Asn Lys Glu Glu Glu Glu Lys Glu Lys Glu Lys Glu Glu Lys Glu Lys
675 680 685

Glu Glu Lys Glu Lys Glu Lys Glu Glu Lys Glu Lys Glu Glu Lys Glu
690 695 700

Lys Glu Glu Lys Glu Lys Glu Glu Lys Glu Glu Glu Lys Lys Glu Lys
705 710 715 720

Glu Glu Glu Gln Glu Glu Glu Glu Glu Glu Glu Ile Val Pro Glu Asn
725 730 735

Leu Thr Thr Glu Glu Ser Lys
740

<210> 54
<211> 1137
<212> DNA
<213> Plasmodium falciparum

<400> 54
ggagaagcag taactccttc cgtaattgat aacatacttt ctaaaattga aaatgaatat 60

gaggttttat atttaaaacc tttagcaggt gtttatagaa gtttaaaaaa acaattagaa 120

aataacgtta tgacatttaa tgttaatgtt aaggatattt taaattcacg atttaataaa 180

cgtgaaaatt tcaaaaatgt tttagaatca gatttaattc catataaaga tttaacatca 240

agtaattatg ttgtcaaaga tccatataaa tttcttaata aagaaaaaag agataaattc 300

ttaagcagtt ataattatat taaggattca atagatacgg atataaattt tgcaaatgat 360

gttcttggat attataaaat attatccgaa aaatataaat cagatttaga ttcaattaaa 420

aaatatatca acgacaaaca aggtgaaaat gagaaatacc ttccctttt aaacaatatt 480

gagaccttat ataaaacagt taatgataaa attgatttat ttgtaattca tttagaagca 540

aaagttctaa attatacata tgagaaatca aacgtagaag ttaaaataaa agaacttaat 600

tacttaaaaa caattcaaga caaattggca gattttaaaa aaaataacaa tttcgttgga 660

attgctgatt tatcaacaga ttataaccat aataacttat tgacaaagtt ccttagtaca 720

ggtatggttt ttgaaaatct tgctaaaacc gttttatcta atttacttga tggaaacttg 780

caaggtatgt taaacatttc acaacaccaa tgcgtaaaaa aacaatgtcc acaaaattct 840

ggatgtttca gacatttaga tgaaagagaa gaatgtaaat gtttattaaa ttacaaacaa 900

gaaggtgata aatgtgttga aaatccaaat cctacttgta acgaaaataa tggtggatgt 960

gatgcagatg ccaaatgtac cgaagaagat tcaggtagca acggaaagaa aatcacatgt 1020

gaatgtacta aacctgattc ttatccactt ttcgatggta ttttctgcag ttcctctaac 1080

ttcttaggaa tatcattctt attaatactc atgttaatat tatacagttt catttaa 1137

<210> 55
<211> 1080
<212> DNA
<213> Plasmodium falciparum

<400> 55
caggataaac ccgaagtaag tgcaaatgat gatacatcac attctacaaa tttgaataat 60

agtttaaaat tatttgaaaa catattgagt cttggaaaaa acaaaaatat ataccaagaa 120

ttaataggtc aaaaaagtag tgaaaacttt tatgaaaaga tattaaaaga tagtgataca 180

ttttataatg aatcttttac aaattttgta aaatctaaag ctgatgatat taattcattg 240

aatgatgaat caaaaaggaa gaaattagaa gaagatatta ataaattaaa aaaaacttta 300

cagttatcat ttgatttata taataaatat aaattaaaat tagaaagatt atttgataaa 360

aagaaaacag ttggtaaata taaaatgcaa attaaaaaac ttactttatt aaaagaacaa 420

ttagaatcaa aattgaattc acttaataac ccaaagcatg tattacaaaa cttttctgtt 480

ttctttaaca aaaaaaaaga agctgaaata gcagaaactg aaaacacatt agaaaacaca 540

aaaatattat tgaaacatta taaaggactt gttaaatatt ataatggtga atcatctcca 600

ttaaaaactt taagtgaaga atcaattcaa acagaagata attatgccag tttagaaaac 660

tttaaagtat taagtaaatt agaaggaaaa ttaaaggata atttaaattt agaaaagaaa 720

aaattatcat acttatcaag tggattacat catttaattg ctgaattaaa agaagtaata 780

aaaaataaaa attatacagg taattctcca agtgaaaata atacggatgt taacaatgca 840

ttagaatctt acaaaaaatt tctcccagaa ggaacagatg ttgcaacagt tgtaagtgaa 900

agtggatccg acacattaga acaaagtcaa ccaaagaaac cagcatcaac tcatgtagga 960

gcagagtcta acacaataac aacatcacaa aatgtcgatg atgaagtaga tgacgtaatc 1020

atagtaccta tatttggaga atccgaagaa gattatgatg atttaggaca agtagtaaca 1080

<210> 56
<211> 660
<212> DNA
<213> Plasmodium falciparum

<400> 56
caggataaac ccgaagtaag tgcaaatgat gatacatcac attctacaaa tttgaataat 60

agtttaaaat tatttgaaaa catattgagt cttggaaaaa acaaaaatat ataccaagaa 120

ttaataggtc aaaaaagtag tgaaaacttt tatgaaaaga tattaaaaga tagtgataca 180

ttttataatg aatcttttac aaattttgta aaatctaaag ctgatgatat taattcattg 240

aatgatgaat caaaaaggaa gaaattagaa gaagatatta ataaattaaa aaaaacttta 300

cagttatcat ttgatttata taataaatat aaattaaaat tagaaagatt atttgataaa 360

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ttctttaaca aaaaaaaaga agctgaaata gcagaaactg aaaacacatt agaaaacaca 540

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<211> 1080
<212> DNA
<213> Plasmodium falciparum

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ttaataggtc aaaaaagtag tgaaaacttt tatgaaaaga tattaaaaga tagtgataca 180

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aaaaataaaa attatacagg taattctcca agtgaaaata atacggatgt taacaatgca 840

ttagaatctt acaaaaaatt tctcccagaa ggaacagatg ttgcaacagt tgtaagtgaa 900

agtggatccg acacattaga acaaagtcaa ccaaagaaac cagcatcaac tcatgtagga 960

gcagagtcta acacaataac aacatcacaa aatgtcgatg atgaagtaga tgacgtaatc 1020

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<211> 1131
<212> DNA
<213> Plasmodium falciparum

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gttatgacat ttaatgttaa tgttaaggat attttaaatt cacgatttaa taaacgtgaa 180

aatttcaaaa atgttttaga atcagattta attccatata aagatttaac atcaagtaat 240

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agttataatt atattaagga ttcaatagat acggatataa attttgcaaa tgatgttctt 360

ggatattata aaatattatc cgaaaaatat aaatcagatt tagattcaat taaaaaatat 420

atcaacgaca aacaaggtga aaatgagaaa taccttccct ttttaaacaa tattgagacc 480

ttatataaaa cagttaatga taaaattgat ttatttgtaa ttcatttaga agcaaaagtt 540

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aaaacaattc aagacaaatt ggcagatttt aaaaaaaata acaatttcgt tggaattgct 660

gatttatcaa cagattataa ccataataac ttattgacaa agttccttag tacaggtatg 720

gtttttgaaa atcttgctaa aaccgtttta tctaatttac ttgatggaaa cttgcaaggt 780

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ttcagacatt tagatgaaag agaagaatgt aaatgtttat taaattacaa acaagaaggt 900

gataaatgtg ttgaaaatcc aaatcctact tgtaacgaaa ataatggtgg atgtgatgca 960

gatgccaaat gtaccgaaga agattcaggt agcaacggaa agaaaatcac atgtgaatgt 1020

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<211> 343
<212> DNA
<213> Plasmodium falciparum

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tgttgaaaat ccaaatccta cttgtaacga aaataatggt ggatgtgatg cagatgccaa 180

atgtaccgaa gaagattcag gtagcaacgg aaagaaaatc acatgtgaat gtactaaacc 240

tgattcttat ccacttttcg atggtatttt ctgcagttcc tctaacttct taggaatatc 300

attcttatta atactcatgt taatattata cagtttcatt taa 343